PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
INDIA
1968

VOL. XXXVIII SECTION—B PART I

Utilization of Monosaccharides by three isolates of *Colletotrichum capsici* (Syd.) Butler and Bisby

By

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[Received on 25th January, 1968]

Various investigators, including Durairaj (1956), Thind and Randhawa (1957, 1957*a*), Misra and Mahmood (1960, 1960*a*), Agnihotri (1961), Chaturvedi (1961), Misra and Dutta (1963), Rai and Chohan (1966) as well as Agnihotri and Prasad (1966) have studied the nutritional requirements of *Colletotrichum capsici*; but very little attention has been paid towards the time taken in the utilization of different monosaccharides. Some of them are most frequently encountered by fungi in nature, either as such or as component units of oligo- and polysaccharides.

In the present study the utilization of monosaccharides by three plant pathogenic isolates of *C. capsici* has been studied chromatographically.

Materials and Methods

Single-spore cultures of *C. capsici* obtained from the infected leaves of *Codiaeum variegatum* (Isolate-A), *Manihot esculenta* (Isolate-B) and *Solanum melongena* (Isolate-C) were employed. They were grown on a number of media and on the basis of the results it was decided to use glucose, 10·0 g; KNO_3, 3·5 g; KH_2PO_4, 1·75 g; $MgSO_4 . 7H_2O$, 0·75 g and distilled water 1000 ml as the basal medium. In order to study the influence of various monosaccharides, *viz.*, L(+) arabinose, D-xylose, L-rhamnose. D-glucose, D-fructose, D-galactose, L-sorbose and D-mannose, they were substituted singly in place of glucose and their quantity was so adjusted as to furnish 4 g of carbon per litre. The most suitable pH for isolates A, B and C was found to be 6·0, 5·0 and 5·5, respectively. The pH of media for subsequent studies was adjusted to the most suitable level in each case. Other methods used were similar to those mentioned by Lal and Tandon (1968).

Results

The dry weight, final pH and time taken for the utilization of monosaccharides have been recorded in table 1.

TABLE 1

Showing average dry weight, change in pH and utilization of different monosaccharides by three isolates of C. capsici.

Sugar	Days of incubation	Isolate–A Dry Wt. mg.	pH	Sugar present days	Isolate–B Dry Wt. mg.	pH	Sugar present days	Isolate–C Dry Wt. mg.	pH	Sugar present days
	5	39·0	6·1		44·6	5·8		27·0	5·8	
Arabinose	10	98·5	8·2	10	98·0	8·2	8	41·6	7·0	15
	15	92·0	8·2		93·0	8·2		80·0	8·2	
	5	38·2	6·1		23·4	6·4		28·9	6·4	
Xylose	10	97·4	7·0	9	43·4	7·3	14	66·0	7·0	14
	15	88·8	7·6		57·8	8·0		76·3	8·2	
	5	20·4	6·4		25·8	5·5		31·8	5·5	
Rhamnose	10	39·4	7·0	15	46·5	6·7	15	76·7	5·8	12
	15	52·6	7·6		66·6	7·6		94·6	7·3	
	5	59·1	6·7		43·3	6·7		49·3	6·7	
Glucose	10	118·5	7·0	7	105·0	7·0	10	102·4	7·0	8
	15	109·4	7·3		98·0	7·3		94·8	7·3	
	5	27·9	5·5		32·5	5·5		42·5	6·7	
Fructose	10	57·1	6·7	15	69·5	6·7	10	75·4	7·0	7
	15	96·5	7·3		64·6	7·3		67·3	7·3	
	5	24·3	6·1		22·7	6·1		24·8	6·1	
Galactose	10	60·9	7·3	14	43·8	7·3	15	56·9	7·0	15
	15	81·6	7·3		73·5	8·2		66·2	7·3	
	5	24·4	6·1		20·0	6·1		23·0	5·8	
Sorbose	10	47·2	6·4	15	45·0	6·4	15	38·9	6·1	15
	15	56·4	7·0		59·2	7·0		43·2	7·0	
	5	49·0	6·1		34·7	5·8		48·5	5·5	
Mannose	10	94·0	6·7	11	98·4	6·1	10	107·4	6·1	11
	15	100·6	7·3		92·0	8·2		101·9	7·0	

Discusion and Conclusion

Table 1 indicates that *C. capsici* isolates A and B utilized arabinose in 10 and 8 days, respectively, while isolate C failed to do so even in 15 days. *Scindapsus pictus* Hassk. isolate of *C. capsici* (Chaturvedi, l.c.) was different in this respect as it took only 7 days to consume this sugar from the medium. Xylose was assimilated

in 9 days by isolate A but other isolates consumed it in 14 days. Another isolate studied by Chaturvedi (l.c.) required 8 days before it could finish the sugar from the medium.

Isolate C could utilize rhamnose in 12 days while the other two isolates of the same species failed to consume it in 15 days. *C. capsici* (Chaturvedi, l.c.) required 11 days for this purpose. The behaviour of isolate A was similar to the isolate studied by Chaturvedi (l.c.) as it took 7 days to utilize glucose from the medium but other two isolates (B and C) finished this sugar in 10 and 8 days, respectively.

Fructose was utilized in 7 and 10 days by isolates C and B respectively but isolate A failed to consume it in 15 days. Only isolate A could finish galactose in 14 days while the other isolates failed to utilize it within 15 days. The behaviour of another isolate studied by Chaturvedi (l.c.) was different in this respect as it took only 7 days to assimilate galactose from the medium.

All the organisms showed poor utilization of sorbose. None of them could consume it in 15 days. Isolate B utilized mannose in 10 days, while the remaining isolates took one day more for this purpose. The isolates of the same species studied by Chaturvedi (l.c.) took 7 days to consume mannose from the medium.

It is thus clear that out of all the monosaccharides, glucose was utilized earlier, while sorbose was consumed very slowly. The rate of utilization of different sugars by various isolates of the same species exhibited marked variation. It is also evident from the above results that there was an increase in dry weight upto the end of the incubation period in all the cases where the rate of assimilation of a sugar was slow but the dry weight in later stages of incubation decreased whenever the sugar was consumed upto 10 days except in isolate C on mannose where a decrease was evident even though it was consumed on the 11th day. The drift in pH showed more or less similar trend. At the end of the incubation period it generally increased and became either neutral or alkaline.

Summary

Utilization of eight monosaccharides, *viz.*, L(+) arabinose, D-xylose, L-rhamnose, D-glucose, D-fructose, D-galactose, L-sorbose and D-mannose, by three isolates of *Colletotrichum capsici* obtained from leaf-spot disease of *Codiaeum variegatum*, *Manihot esculenta* and *Solanum melongena*, respectively, was studied. Chromatographic analysis of the culture medium indicated that glucose was rapidly assimilated by all the isolates, while sorbose was utilized at a very slow rate. Different isolates showed marked variation in the time taken for the utilization of various monosaccharides. In all cases the pH of the media exhibited a drift towards neutrality or alkalinity. In all cases where the sugar was consumed from the medium by 10 days the maximum dry weight was observed on the 11th day ; but generally the dry weight continued to increase where the rate of its utilization was slow. In such cases the maximum weight was observed on the 16th day. The results have been compared with other isolates.

Acknowledgments

The authors are thankful to Prof. D. D. Pant, Head of the Botany Department, for providing the laboratory facilities. They are also thankful to State C. S. I. R. (U. P.) for the financial help to one of us (B. Lal).

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Thind, K. S. and Randhawa, H. S. Studies on the nutrition of fungi—II. The influence of different sources of nitrogen on the growth and sporulation of *Colletotrichum capsici* (Syd.) Butler and Bisby. *Proc. Natl. Acad. Sci.* (India), **27B** : 47–52, 1957*a*.

Proc. Nat. Acad. Sci., India, 38(B), I, 1968

Utilization of Monosaccharides by five isolates of *Colletotrichum gloeosporioides* Penz.

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The time taken in utilization of different monosaccharides by various fungi varies considerably. An attempt has, therefore, been made to undertake chromatographic study of the rate of utilization of various monosaccharides by five isolates of *Colletotrichum gloeosporioides* Penz.

Materials and Methods

The organisms under study were isolated from diseased leaves of *Artocarpus heterophylla* Lam., *Annona squamosa* L., *Mussaenda frondosa* Linn., *Codiaeum variegatum* Blume and *Manihot esculenta* Crantz and in subsequent discussion they have been mentioned as isolates A, B, C, D and E, respectively. Single-spore cultures were prepared with the help of dummy cutter objective. They were grown on a number of media and on that basis it was decided to use glucose, 10·0 g ; KNO_3, 3·5 g ; KH_2PO_4, 1·75 g ; $MgSO_4$. $7H_2O$, 0·75 g ; and distilled water 1000 ml, as the basal medium. In order to study the effect of different monosaccharides they were substituted singly in place of glucose and their quantity was so adjusted as to furnish 4 g of carbon per litre. Eight monosaccharides, *viz.*, L (+) arabinose, D-xylose, L-rhamnose, D-glucose, D-fructose, D-galactose, L-sorbose and D-mannose, were used. The most suitable pH for isolates B, C and E was found to be 6·0 while for A and D it was 5·0 and 5·5 respectively. The pH of media for subsequent studies was adjusted to most suitable level in each case. 25 ml of the medium was taken in each of the 150 ml Pyrex flasks and autoclaved at 15 lbs pressure for 15 minutes. After inoculation with the respective organisms they were incubated at 25 ± 1°C. 0·005 ml of the medium from each set of the flask was analysed every day by circular paper chromatographic technique, described by Ranjan *et al.* (1955). The running solvent was n-butanol-acetic acid-water (4 : 1 : 5) and the spray reagent used was aniline-diphenylamine phosphate (5 vols. 4% aniline, 5 vols. diphenylamine and 1 vol. orthophosphoric acid ; Buchan and Savage, (1952). After spraying, the bands of various sugars were developed by heating the chromatograms at 110°C for 90 seconds. The time taken for the utilization of the monosaccharides has been recorded. The dry weights were also determined and for that purpose the mycelial mats were harvested on previously dried and weighed Whatman No. 42 filter papers after 5, 10 and 15 days. Simultaneously the pH of the filtrate was also determined. The average dry weight of the mycelial mats was taken as criterian for growth. All the experiments were conducted in triplicates.

Results

The dry weight, final pH and time taken for the utilization of monosaccharides have been summarized in table 1.

TABLE 1

Showing average dry weight, change in pH and utilization of different monosaccharides by five isolates of C. gloeosporioides.

Sugar	Days of incubation	Isolate—A Dry wt. mg.	pH	Sugar present days	Isolate—B Dry wt. mg.	pH	Sugar present days	Isolate—C Dry wt. mg.	pH	Sugar present days	Isolate—D Dry wt. mg.	pH	Sugar present days	Isolate—E Dry wt. mg.	pH	Sugar present days
Arabinose	5	25·5	6·4		28·0	5·8		35·0	6·1		20·0	5·8		24·3	6·7	
	10	80·0	7·6	8	68·6	7·3	11	88·0	7·6	7	58·0	8·2	8	48·5	6·7	8
	15	74·3	8·2		75·3	8·2		79·6	8·5		49·0	9·0		40·6	9·0	
Xylose	5	34·3	6·4		25·6	6·4		38·8	6·1		36·9	6·4		45·3	6·4	
	10	85·7	7·3	11	74·5	7·3	11	76·0	7·0	8	82·2	7·0	9	82·1	7·3	10
	15	82·7	7·3		80·8	7·3		70·7	7·3		78·9	7·3		76·7	7·3	
Rhamnose	5	29·4	5·5		20·3	6·1		23·6	6·1		27·2	5·5		19·9	6·1	
	10	67·8	5·5	10	30·0	6·4	15	48·0	6·4	10	46·3	5·8	15	31·1	6·1	15
	15	64·8	7·3		44·7	6·7		46·1	6·7		56·2	6·0		50·3	6·7	
Glucose	5	40·4	6·1		52·8	6·7		67·6	7·0		42·1	6·1		58·4	6·7	
	10	110·4	6·4	8	122·5	7·0	8	122·4	7·0	7	109·1	6·7	8	116·4	7·0	8
	15	98·8	7·3		110·0	7·3		116·0	7·3		99·6	7·3		106·4	7·3	
Fructose	5	30·8	6·1		44·0	6·1		43·0	6·1		41·3	6·1		46·2	6·1	
	10	65·2	6·4	13	62·4	6·4	14	65·4	6·7	13	91·5	7·0	8	69·1	6·7	12
	15	77·7	7·3		79·3	7·3		72·5	7·3		84·0	7·3		88·9	7·3	
Galactose	5	28·3	6·1		40·4	6·1		25·6	6·1		15·0	5·5		23·4	6·1	
	10	42·5	6·7	15	108·4	7·3	12	54·7	6·7	13	45·7	7·0	15	44·7	7·0	15
	15	46·8	7·0		113·2	7·6		78·0	7·3		68·6	7·3		81·0	7·3	
Sorbose	5	35·0	6·1		20·5	6·1		31·4	6·1		23·6	6·1		22·3	6·1	
	10	50·2	6·7	13	41·5	6·1	14	64·3	6·4	12	59·0	6·4	14	56·1	6·1	15
	15	57·6	7·0		58·0	7·0		98·4	7·3		70·4	7·3		66·8	7·0	
Mannose	5	48·4	5·5		53·2	6·1		21·4	6·1		28·4	5·5		34·7	6·1	
	10	110·0	6·7	10	118·5	6·7	10	78·5	7·0	9	74·7	6·7	8	82·3	6·7	10
	15	105·0	7·3		116·6	7·3		74·2	8·2		68·2	7·0		75·4	7·3	

Discussion and conclusion

It is evident from the results that three isolates of *C. gloeosporioides* (Isolates A, D and E) consumed arabinose in 8 days, as has been recorded by Ghosh (1964) for *Carissa* isolate of the same organism. Different isolates of *C. gloeosporioides* studied by Chandra (1961) and Prasad (1963) took 9 days to utilize this sugar. The behaviour of isolates B and C of the same species was different as they could utilize it in 11 and 7 days, respectively.

The isolates A and B took 11 days to assimilate xylose, while corresponding period for isolate E was 10 days only. Isolate-C utilized it in 8 days. Ghosh (*l.c.*) and Singh *et al.* (1965) obtained similar results for their isolates of *C. gloeosporioides*. The behaviour of Isolate-D was similar to the other isolates of the same species studied by Chandra (*l.c.*) and Prasad (*l.c.*).

Isolates A and C finished rhamnose in 10 days, but other isolates of the same species failed to consume it even in 15 days. Eight days were taken for this purpose by the isolates studied by Chandra (*l.c.*) and Chaturvedi (1961). Prasad (*l.c.*) working with another isolate of the same species reported that rhamnose was consumed after 11 days.

Isolate-C utilized glucose fully from the medium after 7 days, while other four isolates needed 8 days to do so. Prasad (*l.c.*) working with *C. gloeosporioides* isolated from 'litchi' mentioned that this sugar was assimilated in 7 days. In this respect his isolate was similar to Isolate-C included in the present study, but the isolates from *Punica granatum* (Chandra, *l.c.*), *Polyscias balfuriana* (Chaturvedi, *l.c.*) and *Mangifera indica* L. (Singh *et al.*, *l.c.*) consumed it earlier and finished it in 6 days.

Isolate-D utilized fructose in 8 days. Similar results were obtained by Chaturvedi (*l.c.*) and Prasad (*l.c.*) with their isolates of the same species. The isolate of this species studied by Chandra (*l.c.*) took 7 days to consume it. Two isolates A and C utilized fructose in 13 days. Corresponding periods for isolates B and E were 14 and 12 days, respectively.

Isolates B and C consumed galactose after 12 and 13 days, respectively, while the remaining three isolates could not utilize it within 15 days. Similar results were obtained by Prasad (*l.c.*) and Singh *et al.* (*l.c.*) with their organisms. The isolates studied by Chandra (*l.c.*) and Chaturvedi (*l.c.*) consumed it faster and finished it in 8 and 11 days, respectively.

Isolates C and A took 12 and 13 days, respectively to utilize sorbose. Corresponding period for isolates B and D was 14 days, while Isolate-E could not utilize it within 15 days. Similar results were obtained by Prasad (*l.c.*) and Ghosh (*l.c.*). The isolate of the same species studied by Singh *et al.* (*l.c.*), consumed it in 10 days only.

Isolate-D exhausted mannose in 8 days, while Isolate-C took a day longer. The remaining three isolates consumed it in 10 days. The time taken by the isolates studied by Chandra (*l.c.*) and Singh *et al.* (*l.c.*) was 11 and 7 days, respectively.

It is thus clear that the rate of utilization of various sugars by different isolates of the same species showed marked variation.

In all those cases where sugars were utilized upto 10 days the dry weights were maximum on the 11th day and they decreased on the 16th day. In other cases there was a continuous increase in dry weight except in the case of Isolate-A on xylose, which showed slight decrease on the 16th day.

The drift in pH in all the cases showed more or less similar trend. At the end of the incubation period it generally increased and became either neutral or slightly alkaline.

Summary

Utilization of eight monosaccharides, *viz.*, L (+) arabinose, D-xylose, L-rhamnose, D-glucose, D-fructose, D-galactose, L-sorbose and D-mannose by five isolates of *Colletotrichum gloeosporioides* Penz. obtained from leaf-spot of *Artocarpus heterophylla, Annona squamosa, Mussaenda frondosa, Codiaeum variegatum* and *Manihot esculenta* was studied. Chromatographic analysis of the medium revealed that glucose was assimilated rapidly, while galactose was utilized slowly by all the isolates. Different isolates exhibited marked variation in the time taken for utilization of various monosaccharides. In all cases the pH of the media showed a drift towards neutrality or alkalinity. In all cases where the sugar was consumed from the medium by 10 days the maximum dry weight was observed on 11th day ; but the weight continued to increase where the rate of its utilization was slow. In such cases the maximum weight was observed on the 16th day. The results have been compared with other isolates of the same species studied by different investigators.

Acknowledgments

The authors are thankful to Prof. D. D. Pant, Head of the Botany Department, for providing the laboratory facilities. They are also thankful to State C. S. I. R. (U. P.) for the financial help to one of us (B. Lal).

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968

Species of *Coemansia* from India

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(With 11 figures)

[Received on 25th January, 1968]

The genus *Coemansia* was first erected by van Tieghem and Le Monnier in 1873. It is one of those Mucorales in which the conidia are borne singly over septate and inflated sporocladia arising from large conidiophores. In the case of *Coemansia*, the conidia are produced on the lower surface of sporocladia as against the genus *Martensella* where the conidia are borne on the upper surface. About sixteen species (Hesseltine, 1956) of the genus have been reported. In India only three species *i.e.*, *Coemansia erecta* Bainier (Rugmini, 1956), *C. reversa* van Tieghem and Le Monnier (Agnihothrudu, 1957) and *C. ceylonensis* Linder (Prasad, 1965) are known. The present paper deals with three isolates which resemble in essential characters with *C. erecta* Bainier, *C. interrupta* Linder and *C. spiralis* Eidam. Out of these three, *C. interrupta* and *C. spiralis* are new reports from India and *C. erecta* was reported by Rugmini (1956) in her thesis only but was never published.

Coemansia erecta Bainier, Bull. Soc. Myc. France **22** : 220-221, 1906 (Plate I, Figs. 1-3).

Colonies growing fast on SMA and Oat meal agar, at first white, becoming bright yellow in age ; mycelium stoloniferous ; conidiophores simple but bifurcate in the upper fertile zone, septate, upto 3 mm in length, 5·2 - 8·7 μ in diameter ; sporocladial stipe one celled, 3·5 - 5 μ long ; sporocladia curved upward from the conidiophore, 4–7 septate, 21–31·5 × 3·5 – 5·2 μ ; terminal cell sterile, straight or curved ; phialides ovoid, 2·2 – 4·5 μ long ; conidia fusoid with apex rounded and base tapering, 6–9·5 × 2·2-3 μ.

Description based on the culture isolated from mouse dung from Allahabad. Culture deposited in BSM Culture Collection, Botany Department, University of Allahabad under No. Mx-100 and also at Centraalbureau voor Schimmelcultures, Baarn, Holland.

Coemansia interrupta Linder, Farlowia, 1 (**1**) : 49-77, 1943. (Plate II, Fig. 1-4).

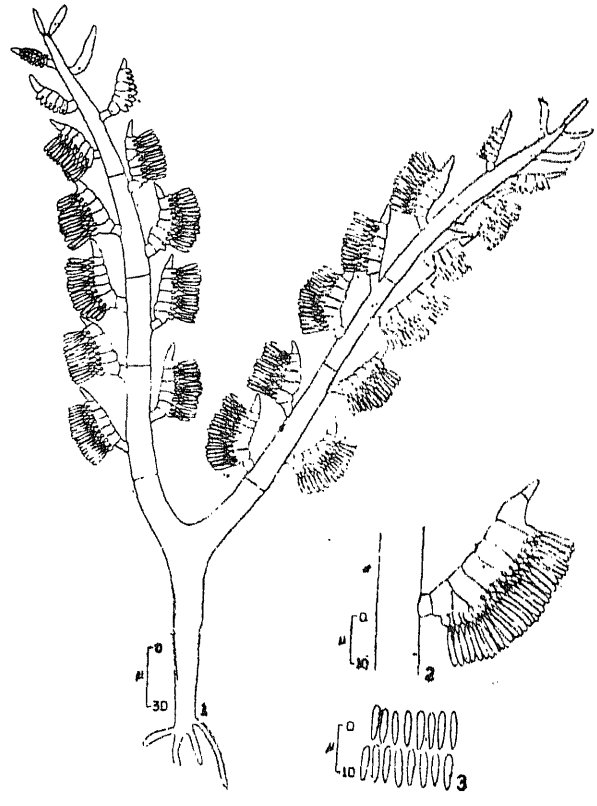
Colonies growing slowly on SMA and Oat meal agar, at first white later becoming pale yellow ; mycelium stoloniferous ; conidiophore erect, septate and regularly bifurcate, 2-3 mm. in length, 9-12 μ in diameter, bearing the sporocladia pleurogenously and close together in clusters of 8-20 ; stipe of sporocladia one-celled, simple or branched, 10·8-24·5 × 5·2–7 μ ; sporocladia 6-8 septate (sometimes 10 septa), 21·6-51·3 × 4·5 10·5 μ, sterile terminal cell short and curved ; phialides elongate, ellipsoid, 3·5-6 × 1·5 μ long ; conidia cylindrical tapering from the middle with pointed ends, 10·5–17·5 × 1·5–2 μ.

Description based on the culture isolated from forest soil of Ranchi. Culture deposited in BSM Culture Collection, Botany Department, University of Allahabad, under No. Mx. 107 and also at Centraalbureau voor Schimmelcutlures, Baarn, Holland.

Coemansia spiralis Eidam, Jahresber. schles, Ges. vaterland. Cult. **64** : 262–263, 1887. (Plate III, Figs. 1-3).

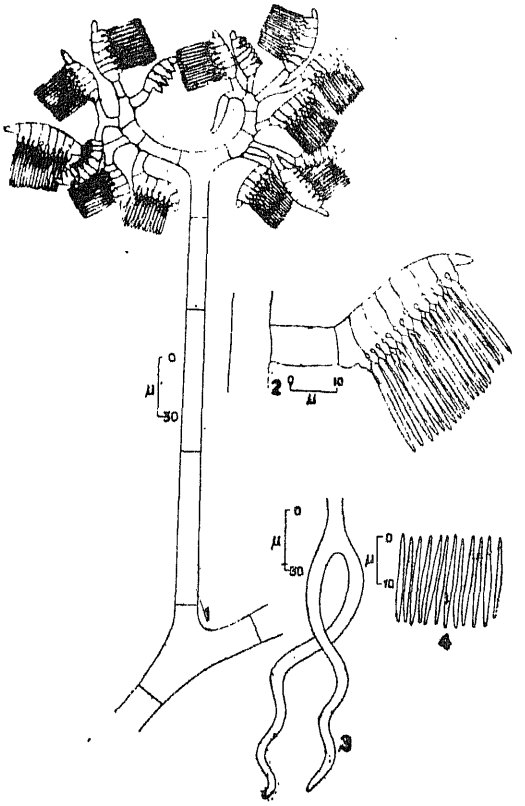
Colonies at first white, becoming cream colour in age ; conidiophores simple, sometimes branched near the substratum, erect, spirally twisted at the tip, 200-750 μ in length, 8–10 μ in diameter, distantly septate ; sporogenous branches stipitate, stipe cells 8-10 μ in length ; sporocladia 3-5 septate, 12·5-25 × 7·0-10·8μ ; sterile terminal cell straight or slightly recurved usually slightly tapered ; phialides numerous on the lower surface, ellipsoid ; conidia cylindrical, tapering to the bluntly rounded base, 10–12·5 × 1·2–1·5 μ.

PLATE 1



Figs. 1-3. *Coemansia erecta* (Camera lucida drawings)
1. A mature conidiophore with basal portion of stolon with rhizoids.
2. Mature sporocladia
3. Conidia

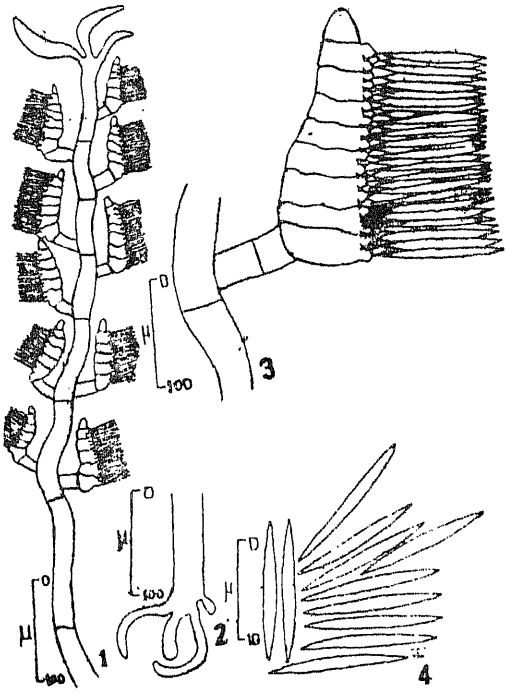
PLATE II



Figs. 1-4. *Coemansia interrupta* (Camera lucida drawings)

1. A branch of the mature conidiophore.
2. A mature sporocladium magnified to show the attachment of the conidia.
3. Basal portion of stolons with rhizoids.
4. Conidia.

PLATE III



Figs. 1-4. *Coemansia spiralis* (Camera lucida drawings)

1. A branch of the mature conidiophore.
2. Lower portion of conidiophore with rhizoids.
3. A mature sporocladium magnified to show the attachment of conidia.
4. Conidia.

The present isolate resembles in essential characteristics (colony characters, spirally twisted conidiophores and size and shape of conidia) of the species given by Eidam (1887) and Bainier (1906). However, it differs in having shorter conidiophores, 200–750 μ in length (according to Eidam, they are 500–1000 μ in length and according to Bainier, they range 1·0-2·0 mm. in length). The sporocladia and the conidia are also smaller in the present isolate. Since the type culture of the species is nowhere available, it seems best to consider the present isolate as a strain of the species of *Coemansia spiralis* Eidam.

Description based on the culture isolated from the soil of Allahabad. Culture deposited in BSM. Culture Collection, Botany Department, University of Allahabad under No. Mx. 110.

Acknowledgement

Thanks are due to Professor D. D. Pant, Head of the Botany Department for the laboratory facilities.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968.

Utilization of Lactose and Formation of synthetic oligosaccharide by isolates of *Botryodiplodia theobromae*[1,2]

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[Received on 29th February, 1968]

In recent years a number of fungi have been found to synthesize oligosaccharides during their growth on various oligo- and polysaccharides. Bacon and Edelman (1950), Blanchard and Albon, (1950) Pazur and French (1952) Giri *et al.* (1953, 1954), Bilgrami (1964) and Ghosh (1966) have presented a detailed account of the synthesis of new transient oligosaccharide, but so far enzymic formation of synthetic oligosaccharide by *B. theobromae* has not been reported on lactose base. The present paper gives a detailed account of the utilization of lactose and formation of a synthetic oligosaccharide from the former by four isolates of *B. theobromae.*

Materials and Methods

Four different isolates of *B. theobromae* obtained from citrus, guava, mango and sapodilla were employed. Basal medium consisted of lactose 10g, KNO_3 3·5g, KH_2PO_4 1·75 g, $MgSO_4$. $7H_2O$ 0·75g and one litre of redistilled water. 25ml of the basal medium apportioned in 150 ml conical Erlenmeyer Pyrex flasks were steam-sterilized for three consecutive days for half an hour. Flasks containing culture medium were inoculated with pure monoconidial cultures of the different isolates of *B. theobromae* separately and incubated at 25±1°C for 15 days. Daily analysis of the culture filtrate was carried out by circular paper chromatography. The general procedures were similar to those followed earlier by Srivastava and Tandon (1966-67). Dry weight of the fungal mat after 5, 10 and 15 days of incubation was taken as the growth criterion.

Results

The results obtained have been summarized in Tables 1 and 2.

TABLE 1

Showing average dry weight (in mg) of the mycelial mats of four isolates of B. theobromae *on lactose and its component hydrolytic products.*

Sugar	Days of incubation	Citrus isolate	Guava isolate	Mango isolate	Sapodilla isolate
	5	25·3	24·3	27·0	43·6
Lactose	10	72·0	75·3	49·6	55·6
	15	83·0	93·6	59·0	64·6
D-glucose	5	32·2	30·2	29·2	35·0
+	10	81·2	75·6	73·8	79·4
D-galactose	15	72·2	70·2	81·2	89·0

1. Part of the first author's Ph.D. thesis submitted to the University of Allahabad.

2. This research was financed in part by a grant made by United States Department of Agriculture, Agricultural Research Service under the head PL 480.

TABLE 2

Showing the presence (in days) of lactose and synthetic oligosaccharide during the growth of four isolates of B. theobromae.

Sugar	Synthetic product	Citrus isolate	Guava isolate	Mango isolate	Sapodilla isolate
Lactose		0-15	0-15	0-15	0-15
	Oligosaccharide (Rf. 0·15)	5-15	4-15	5-15	9-15
D-glucose + D-galactose		1-6	1-4	1-6	1-7
		1-9	1-10	1-13	1-12

Discussion

It is obvious from the results that none of the isolates could consume lactose completely and it persisted in the medium upto the end of the incubation period. Slow utilization of lactose has also been reported by various investigators working with species of *Botryodiplodia* and other fungi including species of *Calvatia* (Sedlmayr, 1961), *Fusarium solani*, *Macrophomina phaseoli*, *Botryodiplodia ananassae* (Bhargava, 1962), *B. theobromae*, *Pestalotia pauciseta* and *Colletotrichum gloeosporioides* (Prasad, 1965). All these fungi utilized this sugar through non-hydrolytic pathway, although Kakkar (1964) observed the appearance of synthetic oligosaccharide in the medium used by fungi investigated by him. In the present investigation a synthetic oligosaccharide appeared after 4 or 5 days of incubation in the medium used by all the isolates, except sapodilla isolate where it appeared after 9 days of incubation. In all cases the oligosaccharide persisted throughout the incubation period. Formation of such oligosaccharide in the medium is indicative of the hydrolysis of lactose and thus its utilization through hydrolytic pathway. Partial hydrolysis of the synthetic oligosaccharide yielded lactose and galactose. Formation of synthetic oligosaccharide may be attributed to the activity of the enzyme transgalactosidase which might be responsible for transferring galactose residue to lactose in the formation of this trisaccharide. Since no detailed study could be made regarding the chemical identification of the synthetic oligosaccharides it has been considered desirable to refer them with their Rf. values.

A comparison of the dry weight of mycelial mat on lactose with that on a mixture of glucose and galactose revealed that citrus and guava isolates attained better growth on lactose than on the equimixture of its hydrolytic products. The case was just the reverse for mango and sapodilla isolates.

Summary

Utilization of lactose by four fruit rotting isolates was studied. Lactose was utilized by all the isolates of *B. theobromae* apparently through a non-hydrolytic pathway with the formation of a synthetic oligosaccharide having a Rf. value of 0·15 in all the cases. Enzymic synthesis of an oligosaccharide during the utilization of lactose by isolates of *B. theobromae* is being reported for the first time.

Acknowledgement

The authors are greatful to Prof. D. D. Pant for providing necessary laboratory facilities.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968.

Studies in the Leguminosae

1. Male and female gametophytes of *Indigofera enneaphylla* Linn.

By

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[Received on 29th February, 1968]

Introduction

Indigofera enneaphylla Linn. is an annual, trailing, much branched herb, with pinnately compound leaves. Leaf-lets are hairy all over and silvery beneath. The flowers are produced in small clusters. The pod is oblong and thinly silky, often 2-seeded, rarely one or three seeded.

Material and Method

The material was collected locally and was fixed in Formalin-acetic alcohol. Dehydration and embedding was done as per usual methods. The sections were cut 10 to 12 μ thick and stained with Heidenhain's iron-haematoxylin. Erythorsin was used as a counterstain.

Microsporogenesis and Male Gametophyte

The floral whorls differentiate in acropetal order. The undifferentiated anther consists of a mass of homogeneous cells. It becomes four lobed as the archesporium differentiates. It is limited to a row of hypodermal cells in each lobe. (Fig. 1).

The epidermal cells divide anticlinally during the early development of the anther. As the internal tissues increase in amount the cells of the epidermis undergo a change in shape becoming radially flattened and stretched. These cells, prior to dehiscence, form a thin layer of dead cells over the endothecium. (Figs. 7-10).

The archesporial cells divide periclinally to form an outer primary parietal cell and an inner primary sporogenus cell (Fig. 2). The parietal cell, by further periclinal and anticlinal divisions, gives rise to the endothecium, middle layer and the tapetum. (Figs. 3 and 4). These cells, when first formed, are similar in size but gradually change their shape and size.

The outermost layer, below the epidermis, differentiates into endothecium. Prior to the dehiscence a stomium originates as a lateral depression along the middle line of the thecae (Figs. 11, 11A). The stomial furrow deepens as the epidermal and hypodermal cells enlarge radially. The hypodermal cells later develop characteristic secondary fibrous thickenings (Figs. 7, 8, 9, 12, 12A). The adjacent pollen sacs of two thecae fuse along their length (Fig. 14). The fusion is brought about by the disorganization of the intervening septa (Figs. 13, 13A). The parenchymatous cells in the region of the septa are differentiated into small parenchymatous cells situated at the base of the stomial groove while the cells

PLATE 1

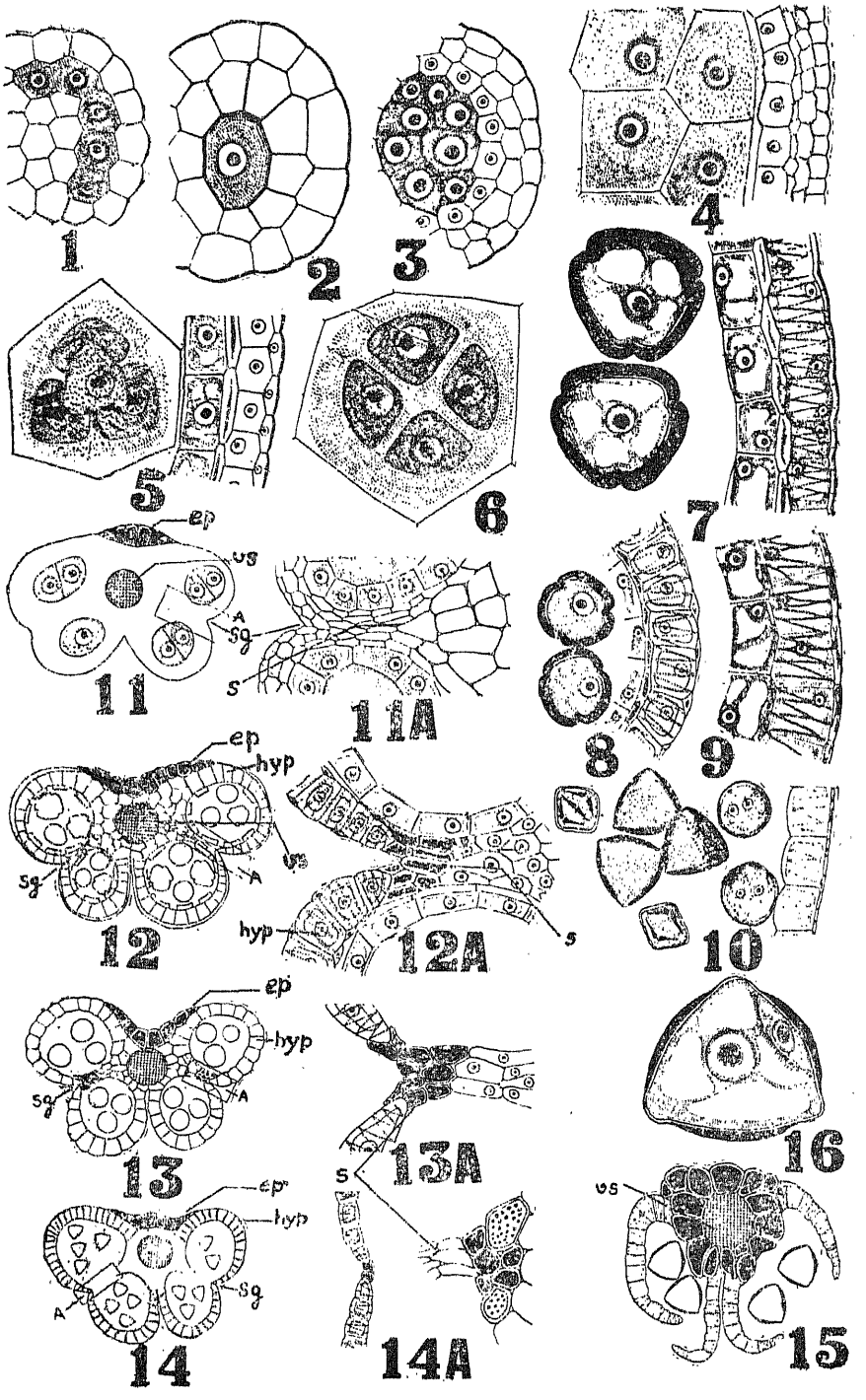
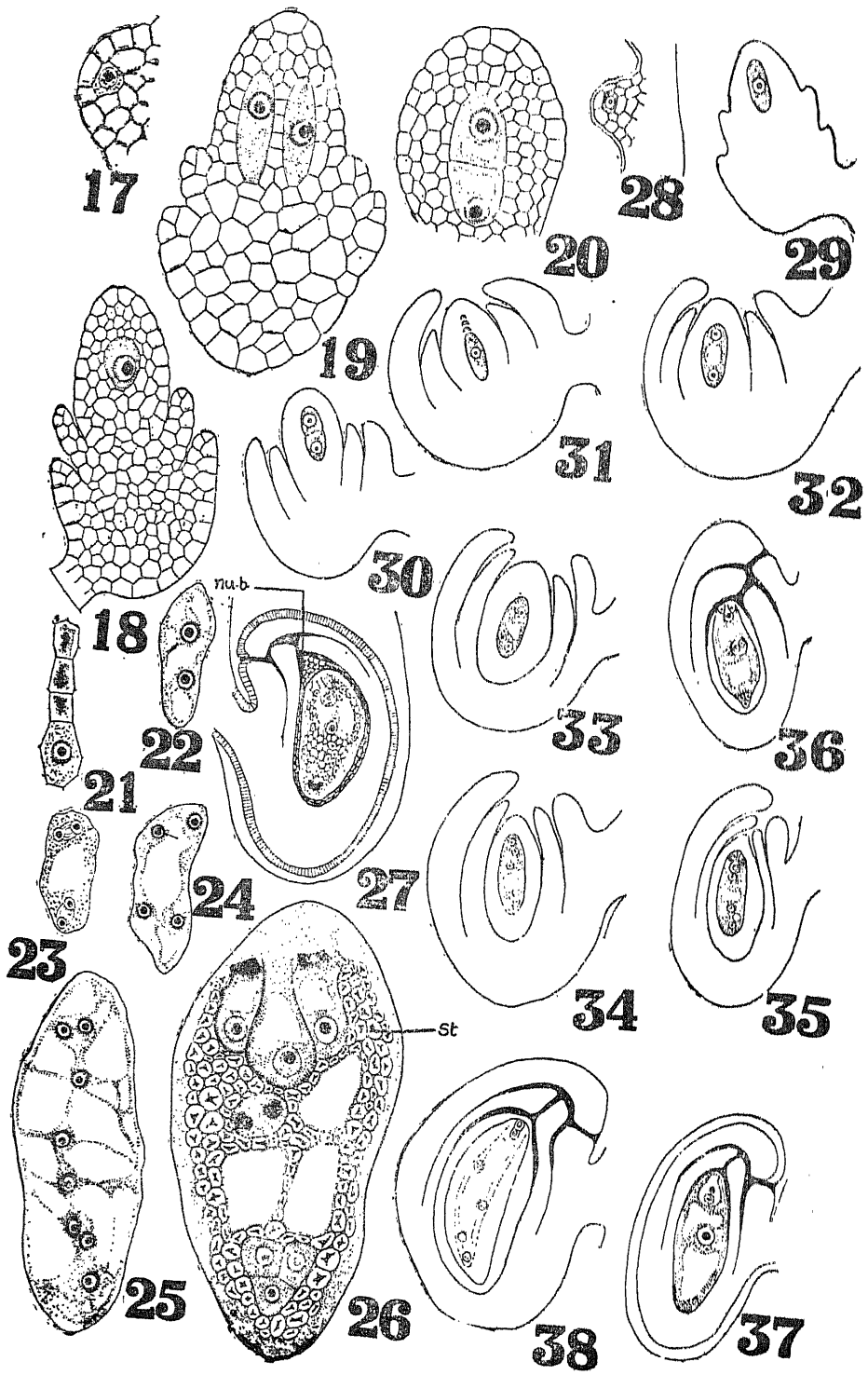


PLATE 2



EXPLANATION TO FIGURES

PLATE 1

Indigofera enneaphylla. Linn. (Fig. 1-6 ; *ep.*—epidermis ; *s.*—septum ; *sg.*—stomial groove ; *t*—tapetum ; *vs*—vascular supply.)

Fig. 1—L. S. Young anther showing male archesporial cell. Fig. 2—T. S. Young anther showing a parietal layer and sporogenous cell Figs. 3 and 3 – T. S. anther showing stages in the development of anther. Fig. 5—T. S. anther showing tepetum, degenerating middle layer, endothecium and epidermis ; note tetrahedral microspore tetrad. Fig. 6—Isobilateral tetrad of microspores. Fig. 7 to 10—T. S. mature anther : note vacuolated tapetal cells and disappearance of inner tangential wall ; fibrous endothecium, persistant middle layer and mature pollen grains of two sizes. Figs. 11 to 15— L. S. anther showing stages in the anther dehiscence Figs. 11A to 14A—*magnified portions of* part 'A' of figs 11 to 14. Fig. 16—mature pollen grains.

(Figs. 1 to 7 and 9—X800 ; Figs. 8 and 11A—X500 ; Fig. 10—X650 ; Fig. 11, 12A 13A ; 14A,—X 225, Figs. 12, 13, and 15—X125 ; Fig. 14—X75.)

PLATE 2

Indigofera enneaphylla, Linn.(Figs 17 to 33 *nu. b.*—nucellar beak ; *st.*—starch).

Fig. 17 —L. S. ovule primordium showing hypodermal female archesporium ; Figs. 18 and 19—L. S. young ovule showing megaspore mother cells (note initiation of integuments and two megaspore mother cells in Fig. 19 ; Fig. 20—L.S ovule showing a dyad : Fig. 21—Linear tetrad of megaspores, with three degenerating micropylar megaspores. Figs. 22 to 25—2, 4 and 8 nucleate embryo sacs. Fig. 26—mature embryo sac ; note the starch grains and filiform apparatus. Fig. 27 —L. S. ovule showing micropyle (note zigzag micropyle formed by both the integuments and a nucellar beak at the apex of embryo sac. Figs. 28 to 38—Stages in the development of ovule.

(Figs. 17, 18 and 19—X500 ; Figs. 20 to 26—X800 ; Figs. 27, 30 to 33—X225 ; Figs. 28 and 29—X300 ; Figs. 34 to 37—X125.)

towards the connective are large (Fig. 11A). The cells below the stomium undergo dissolution first (Figs. 13, 13A) to form a narrow passage. This passage gradually widens as more and more parenchyma forming the septa becomes involved in this process. (Figs. 14, 14A). Vascular tissue is strengthened by the sclerification of the surrounding cells. (Fig. 15).

The actual dehiscence involves the rupture of the anther wall along the stomium. The anther wall along the stomium spreads apart due to the mechanical action of the thickened epidermal cells and fibrous layer and the initial narrow opening becomes wider. The curling edges of the wall (Fig. 15) result in two, long linear slits along the thecae.

Middle layer in this plant persists for a while as flattened cells even after the tapetum has been completely absorbed and uninucleate pollen grains have been formed. (Fig. 8). However, at dehiscence no trace of these cells is seen. (Fig. 10, 13, 14).

The cells of the tapetum become flattened, (Figs. 3, 4). They increase in size and in their staining capacity. At the microspore tetrad stage small vacuoles appear in these cells (Fig. 5). As the microspores separate from each other the inner radial walls of the tapetal cells breakdown (Figs. 8, 9) and some chemical substance flows out into the anther loculus and comes in contact with the developing microspores. The tapetal cells remain uninucleate throughout and the contents remain *in situ* (Figs. 7, 8. 9). Ultimately these are absorbed by the developing pollen grains (Fig. 10). Thus the tapetum conforms to the secretory type.

The primary sporogenous cells undergo three mitotic divisions to develop into microspore mother cells (Fig. 3). These divide meiotically forming four pollen grains. Cytokinesis is of the simultaneous type and occurs by furrowing (Figs. 5, 6). The microspore tetrads may be tetrahedral or decussate (Figs. 5, 6).

The tetrads remain enclosed within a membrane of microspore mother cells. The membrane is thick and gelatinous and microspores remain together in gelatinous matrix for some time. The enclosing walls later get absorbed and the microspores are liberated free into the pollen sac (Figs. 7, 8, 10).

The microspore, to begin with, has dense cytoplasm. As it increased in size, vacuoles begin to appear (Figs. 7, 8). The pollen grains occur in two sizes (Fig. 10). They are spheroidal in shape and are tri-zonicolpate (Fig. 10). The exine is thick (Fig. 16). The pollen grains, at anthesis are bi-celled (Fig. 10). The vegetative cell consists of a large nucleus surrounded by thin layer of vacuolated cytoplasm (Fig. 16).

Megasporogenesis and Female Gametophyte

The gynoecium consist of a monocarpellary, unilocular superior ovary with usually two ovules. Sometimes there are three ovules. Ovules are campylotropous, bitegmic and crassinucellate. To begin with, the ovule primordium inside the ovary grows straight towards the dorsal suture (Fig. 28). It curves upwards even before the integument initials appear. The curvature is due to vigerous growth between hilum and chalaza and the curvature becomes more pronounced by the difference in growth on the two sides of the outer integument (Figs. 29–37) while the growth of inner integument is more or less uniform. The growth of the dorsal half of outer integument is more vigerous. According to Reeves (1930) the ovule grows straight till it meets the dorsal wall of the carpel and thus curvature is due to interference of the carpellary wall. However, in *I. enneaphylla* ovule primordium begins to curve before it comes in contact with the dorsal wall of the carpel. As a result of differential growth the ovule assumes compylotropous form (Fig. 37).

The primordium of the integument differentiates simultaneously with the differentiation of the megaspore mother cell (Fig. 29). They arise at the base of the nucellus and arise from the nucellar epidermis in acropetal succession (Fig. 29). The integuments, to begin with, are two celled thick over their entire length except at the apex where the outer integument is slightly thickened (Figs. 36, 37). The massive outer integument overgrows the inner (Fig. 32) but later both take part in the formation of a zigzag micropyle (Figs. 37, 38). The epidermal cells of the outer integument become columnar.

The archesporium is hypodermal in origin (Fig. 17). It cuts off a primary parietal cell and a primary sporogenous cell. The anticlinal and periclinal divisions of the primary parietal cell result in the formation of a parietal tissue of 2 to 3 cells thick, (Figs. 18, 19). The primary sporogenous cell increases in size and becomes the megaspore mother cell (Fig. 18). Occasionally there are two megaspore mother cells (Fig. 19) as in *Medicago sativa* (Martin, 1914; Reeves, 1930). It undergoes usual meiosis resulting in a dyad (Fig. 20) and finally a linear tetrad of megaspores is formed, (Fig. 21). Only the chalazal megaspore functions, while the remaining three degenerate (Fig. 21).

The functional megaspore undergoes increase in size. Its nucleus divides to form two daughter nuclei and a vacuole is formed in between them. Thus the daughter nuclei move apart form each other towards the ends of embryosac (Fig. 22). The nuclei at the opposite poles undergo two mitotic divisions and eight nuclei thus formed are arranged into two groups of four each (Figs. 23, 24, 25). The cytoplasmic cleavage results in the formation of three uninucleate cells at each pole and two nuclei in the centre of the embryo sac (Fig. 25). The development of the embryo sac thus conforms to the Polygonum type. (Maheshwari, 1950). During development embryo sac increases enormously in size (Fig. 22–26).

The three uninucleate cells at the micropylar end of the embryo sac form the egg apparatus, which consists of two synergids and egg (Fig. 26). The synergids are pear shaped and hooked. At the advanced stage they show filiform apparatus (Fig. 26). The broad base of the synergids obscure the egg. The egg has a dense basal cytoplasm with a nucleus, while the micropylar end is occupied by a conspicuous vacuole (Fig. 26).

The central portion of the embryo sac is occupied by a secondary nucleus formed by the fusion of two poler nuclei. The secondary nucleus lies below the egg (Fig. 26).

There are three antipodals (Fig. 20) situated at the chalazal end of the embryo sac. They are uninucleate and degenerate soon after their formation.

During the development of the embryo sac, the nucellar tissue surrounding the embryo sac, with exception of nucellar cap breaks down. The nucellar cap forms a plug between the apex of female gametophyte and the micropyle(Fig. 27).

The mature embryo sac is lanceolate and is completely filled with starch grains which, however, disappear after fertilization.

Discussion

The male archesporial cell in *I. enneaphylla*, as in other members of the Leguminosae, divides normally into primary wall cell and the primary sporogenous cell. The former on further divisions produces a wall tissue consisting of three layers of cells. However, wall tissue may be of varying thickness. In *Albizzia lebbek* (Maheshwari, 1931) it consists of 6 to 7 layers of cells, while wall cells have been reported to be absent in *Lathyrus odoratus* (Johanson*) and *Orobus angustifolius* (Guignard*).

The endothecium shows usual fibrous thickenings and the epidermis in *I. enneaphylla* forms a thin layer of dead cells over the endothecium as in *Trifolium pratense* (Hindmarsh, 1964). The middle layers in the members of the Leguminosae generally degenerate at an early stage of development. However, in the species under investigation it persists for a considerable time and is seen in the form of a narrow band of cells even at the uninucleate stage of the pollen grains.

The cells of the tapetum remain uninucleate (Weistein, 1926 ; Latter, 1926 ; Reeves, 1930 ; Cooper, 1933 ; Castetter, 1926 ; Pantulu, 1945) Tapetal cells may become biseriate at places in *Millettia ovalifolia* (Pal, 1960). Uninucleate tapetal cells appear to be common feature in the Papilionatae. Tapetal cells in *Cassia didymotorya* (Sethi, 1930) and *Cassia tora* (Datta, 1934) are binucleate. Ghose and Alagh (1933) reported bi-, tri-nucleate tapetal cells and also observed 6 or 7 nuclei in certain cases. The pollen grains in the Papilionaceae are shed at two celled condition. However, according to Pal (1960) they are shed at three-nucleate stage.

The integument primordia appear simultaneously with the formation of megaspore mother cell. However, according to Samal (1936) in *Crotolaria juncea*, and Pal (1960) in *Millettia ovalifolia* primordia of the integument arise simultaneously with differentiation of archesporial cell, but this may be an error due to the observation of a old material.

The inner integument, as in other members of the Papilionaeceae, in *I. enneaphylla*, appears slightly before that of the other. In *Millettia ovalifolia* (Pal, 1960) and *Alycicarpus longifolius* (Purhekar, unpublished) the outer integument overgrows the inner and alone forms the micropyle. In *I. enneaphylla* both the integuments are well developed and take part in the formation of a zigzag

*Quoted from Dahlgren.

micropyle. In *Cajanus indicus* and *Lathyrus sativus* (Roy, 1933) inner integument is much less developed than the outer. In *Acacia baileyana* (Newman, 1933) integuments are poorly developed and ovule consequently is almost naked.

Archesporial cell in the ovule is unicellular and hypodermal in *I. enneaphylla* as in other Papilionaceae. (Maheshwari, 1931 ; Ghose and Alagh, 1933 ; Newman, 1933 ; Singh and Shivpuri, 1935 ; Paul, 1937 ; and Pantulu, 1945). Multicellular archesporium has been reported in the Papilionaceae by Martin (1914), Brown (1917), and Reeves (1930). Deep seated archesporial cell in subhypodermal layer has been reported by Saxton (1907), Datta (1934), Roy (1933) and Dnyansagar (1949) and this according to them may function directly as megaspore mother cell. The deep seated origin of archesporium appears to be rather incorrect and where it is described subhypodermal, it is likely that the same is hypodermal.

The tetrads of megaspores is linear in *I. enneaphylla.* In *Crotolaria juncea* (Samal, 1936) and sometimes in *Arachis hypogea* (Smith, 1956) tetrad is T-shaped. Occasionally the tetrad is isobilateral or T-shaped in *Cassia glauca* (Pantulu, 1945).

The chalazal megaspore usually functions and develops into as embryo sac. However, variations are found in the Leguminosae. According to Guignard (1881) the megaspore, next to the chalazal end is functional. Similar situation was reported in *Cassia tomentosa* (Saxton, 1907) and *Cassia purpurea* (Ghose and Alagh, 1933). Variations may occur in the same genus, *viz. Cassia* (Datta, 1935) or in the same species, *viz., A. lebbek* (Maheshwari, 1931).

The nucellus disintegrates during development and the embryo sac comes in direct contact with inner integument (Brown, 1917 ; Cooper, 1935 ; and Farley and Hutchinson, 1947). In *I. enneaphylla* nucellar epidermis remains over the apex of the gametophyte as in *P. multiflores* (Guignard, 1881) and *Phaseolous vulgaris* (Wiestein, 1926). However, this apical layer of nucellus is multiseriate in *Acacia* (Guignard, 1881 ; Newman, 1934).

The embryo sac development in the Leguminosae conforms to the normal type. Variations in development have been reported. In *Medicago arborea* (Herail, 1889*) it is of the bisporic type, while in *Melilotus alba* (Young, 1905) megaspore mother cell acts as a megaspore. However, according to Coe and Martin (1920) megaspore mother cell in *Medicago alba* does divide and embryo sac development proceeds in a normal manner. In *Lathyrus odoratus* (Jonsson, 1879–80) embryo sac development is of Scilla type, but in *L. sativus* (Roy, 1933) it is of the normal type. The investigations show that the embryo sac development in the Leguminosae is of normal type and the abberent types worked out long ago require confirmation.

The antipodals in *I. enneaphylla* as in other members of the Papilionaceae degenerate at an early stage of development, however, in *Millettia ovalifolia* (Pal, 1960) antipodals degenerate rather slowly and their remanents could be found long after fertilization.

The embryo sac is filled with starch. The starch grains appear at the 4-nucleate stage in *Medicago sativa* (Cooper, 1935) and at the functioning megaspore stage in *Acacia* (Guignard, 1881). However, as in *Arachis hypogea* (Smith, 1956), starch grains in *I. enneaphylla* are not formed until 8 cells of the embryo sac has become differentiated. The starch grains in this plant disappear after fertilization, while in *Acacia baileyana* (Newman, 1931) starch grains remain present even during endosperm formation.

*Referred by Reeves.

Summary

Development of male and female gametophytes in *I. enneaphylla*, Linn. is described in detail.

The primary archesporium is hypodermal in origin. It consists of single row of cells in each anther.

The middle layer persists for a while. The endothecium develops usual fibrous thickenings.

The tapetum is the innermost derivative of the parietal cell. It is of a secretory type. Its cells remain uninucleate.

The primary sporogenous cells undergo mitotic divisions.

Cytokienesis is of simultaneous type and occurs by furrowing.

The microspore tetrads may be tetrahedral or isobilateral.

The pollen grains are spheroidal, tri-zonicolpate. They are shed at 2-celled stage.

The ovules are campylotropous, crassinucellate and bitegmic. The micropyle is formed by both the integuments.

The archesporial cell is hypodermal, and cuts of parietal cell. The parietal tissue consists of 3 to 4 layers of cells. Parietal tissue, during development of embryo sac, is destroyed except at the apex, where it forms a plug between apex of female gametophyte and micropyle.

The magaspore mother cell gives rise to a linear tatrad of megaspores. The chalazal megaspore functions. The embryo sac development conforms to the Polygonum type.

The synergids are hooked. Filiform apparatus is present.

The antipodals degenerate early.

The embryo sac is filled with starch.

Acknowledgement

We are indebted to Prof. L. B. Kajale for encouragement and facilities.

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Proc. Nat Acad Sci., India, 38(B), I, 1968

Comparative Efficiency of Different Nitrogenous Fertilizers in Relation to yield of Cotton Crop

By

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[Received on 26th July, 1967]

Introduction

Nitrogen is the chief limiting factor in the production of high yields of cotton crop in Uttar Pradesh. It has been fully established that the application of this nutrient element through ammonium sulphate causes significant enhancement in the yield of *Kapas* (Dabral 1955, Sharma 1961, Singh *et al.*, 1966). But ammonium sulphate is in extremly short supply in comparison to the heavy demand for it. Moreover, considering the cost per kg. of nitrogen in it, this fertilizer is also more costly than other inorganic nitrogenous fertilizers. Hence, there was necessity of conducting the present studies with a view to find out a cheaper but equally efficient source of inorganic nitrogen to be used as a substitute for ammonium sulphate in cotton crop.

Review of literature

Skinner and Buie (1926), Andrews (1947) and Collings (1947) reported that ammonium chloride is almost as effective a source of nitrogen to crops like cotton, potato and tobacco as ammonium sulphate. Mehta and Jaisinghani (1962) found that ammonium sulphate, calcium ammonium nitrate and urea are equally effective for cotton crop in red loam soils of Rajasthan. Chavda *et al.* (1963) claimed better results with chilean nitrate than with ammonium sulphate, urea, calcium cynamide and ammonium sulphate nitrate in the black cotton soils of South Gujarat. Garg (1964) failed to observe any difference between the efficiency of urea and calcium ammanium nitrate. Dargan *et al.* (1965) recorded significantly higher yield with chilean nitrate than with calcium ammonium nitrate in the first year of his trial, while in the second year, calcium ammonium nitrate fared better than the other fertilizers. In the pooled results of the two years, however, chilean nitrate proved significantly superior to calcium ammonium nitrate, urea and ammonium sulphate but was at par with ammonium sulphate nitrate.

Material and methods

The experiment was conducted under field condition during the crop seasons 1964–65 and 1965-66. The soil of the experimental fields was sandy loam with 7·4 pH and had 0·55% organic carbon, 0·0614% total nitrogen and 0·0146% available nitrogen in it.

The experiment had a randomised block design with 4 replications and with 6 treatments as detailed in table I. The net plot size was 11·9 sq. m. and the American cotton variety, 320F, was sown in the experiment.

The experimental crops were sown in the middle of April and given one irrigation about 6 weeks after sowing in both the seasons. The different fertilizers were applied at 35 Kg. N/ha. in two equal instalments, one at sowing time and the other at flowering time. All other cultural operations were given to the crops according to the normal recommended practice.

Results and discussion

1. *Kapas yield* :

The yields of *Kapas* obtained from various treatments are presented in table I.

TABLE I

Yield of Kapas (Kg./ha.)

Treatments	1964–65	1965–66	Mean	% increase over control
Control (No manure)	1043·8	1454·1	1248·9	–
Ammonium sulphate	1163·7	1725·6	1444·7	15·7
Calcium ammonium nitrate	1306·8	1630·9	1468·9	17·6
Ammonium sulphate nitrate	1260·5	1607·7	1434·1	14·8
Ammonium chloride	1281·6	1748·6	1515·1	21·3
Urea	1170·0	1620·0	1395·0	11·7
S. E.	±117·3	±74·6		
C. D. at 5%	Not sig.	224·8		

It is observed from this table that, with the application of 35 kg. N/ha through various sources, the *kapas* yields were enhanced in both the seasons, but the beneficial effect of different fertilizer treatments over control was clearly marked only during the season 1965–66. Considering the mean yields of two seasons, ammonium sulphate, calcium ammonium nitrate, ammonium sulphate nitrate, ammonium chloride and urea produced 15·7%, 17·6%, 14·8%, 21·3% and 11·7% increased yields respectively over control.

2. *Economics* :

The economics of different treatments, worked out on the basis of costs involved due to application of various fertilizers, and the increased yields resulting therefrom are presented in table II. The prices of ammonium sulphate, calcium ammonium nitrate, ammonium sulphate nitrate, ammonium chloride and urea have been calculated at Rs. 366, Rs. 346, Rs. 438, Rs. 400 and Rs. 615 per M. T. respectively and the selling rates of *kapas* for the seasons 1964-65 and 1965-66 @ Rs. 117 and Rs. 125 per quintal respectively.

TABLE II

Economics of different treatments

Treatments	Additional yield over control Kg./ha. 1964-65	1965-66	Value of additional produce Rs./ha. 1964-65	1965-66	Mean value of additional produce Rs./ha.	Cost of fertilizer and its application Rs./ha.	Net profit Rs./ha	Net profit per Rupee invested
Control	–	–	–	–	–	–	–	–
Ammonium sulphate	119·9	271·5	140·28	339·37	239·83	66·05	173·78	2·63
Calcium ammonium nitrate	263·0	176·8	307·71	221·00	264·35	62·55	201·80	3·23
Ammonium sulphate nitrate	216·7	153·6	253·54	192·00	222·77	58·80	163·97	2·79
Ammonium chloride	237·8	294·5	278·23	368·13	323·18	56 00	267·18	4·77
Urea	126·2	165·9	147·65	207·37	177·51	52·15	125·36	2 40

The figures given in the above table show that, from economic point of view, ammonium chloride was the best fertilizer for cotton crop because every rupee invested on it gave the highest net profit of Rs. 4·77.

Summary and Conclusions

An experiment was conducted with American cotton, 320 F, for determining the comparative efficiency of ammonium sulphate, calcium ammonium nitrate, ammonium sulphate nitrate, ammonium chloride and urea. All of these fertilizers, when applied at a uniform level of 35 Kg. N/ha., proved almost equally effective in increasing the yield of cotton crop. From economics point of view, however, ammonium chloride was found to be more profitable than other fertilizers. It is, therefore, advisable to use ammonium chloride in place of ammonium sulphate for fertilizing cotton crop in Uttar Pradesh.

Acknowledgements

The authors are grateful to the Indian Central Cotton Committee for financing the Scheme for Agronomic and Physiological Research on Cotton in Uttar Pradesh under which the present invastigations were conducted. Their thanks are also due to Dr. Hari G. Singh, Economic Botanist (Cotton & Tobacco), to Govt. U. P., Bulandshahr for providing necessary facilities for these investigations.

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Proc. Nat. Acad. Sci., India, 38 (B), I, 1968

Studies on the Effect of Topping on Yields of Desi and American Cottons in Uttar Pradesh

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Introduction

In cotton, topping implies the pruning of the terminal growing points of stems and branches. In the early stage, it is done in order to check linear growth and to accelerate lateral branching, while in the late stage, it is resorted to for preventing vegetative growth and for inducing earliness in the plant.

There are conflicting views of different workers about the usefulness of topping. Redding and Kimbrough (1906), in Georgia, reported that topping is not advisable for it does not pay. Ayres (1921), in Mississippi, observed that topping decreased yield, more so when it was done at an early stage. Templeton (1931) also found that there was a deleterious effect of topping on yield. Christidis and Harrison (1955) reported on the basis of over twenty experiments conducted in Greece from 1934 to 1940 that topping was in no case better than no topping either in yield or earliness in maturity. It instead depressed the yield particularly when carried out rather early. Therefore, they were of the view that this practice may profitably be omitted altogether so as to save on labour as well as to avoid possible reduction in yield. Bennett *et al.* (1965) found that lodging was almost eliminated and boll rot reduced by topping or by a combination of topping and pruning both. According to them, topping at 36, 42 or 48 inches or pruning also brought about a substantial increase in yield. Dargan *et al.* (1965) reported that topping depressed values of almost all the plant characters including the final yield and resulted in a loss of income from the crop.

With a view to assessing the usefulness of topping in cotton crop in Uttar Pradesh, investigations were carried out at Government Cotton Research Station, Raya (Mathura), the results of which are reported in this article.

Materials and method

Two field experiments were laid out, one with American cotton variety, 320F (*G. hirsutum*) and the other with Desi cotton variety, Raniben (*G. arboreum*), during each of the seasons 1964-65 and 1965-66. The layout of these experiments was in a randomised block design with four treatments as detailed in table I and the number of replications was six in each case. The net plot size of these experiments was 12·01 sq. metres in 1964-65 and 11·52 sq. metres in 1965-66. All cultural, manurial and irrigational operations except the topping treatments were uniformly given to the whole crop. The yearwise *kapas* yield data were statistically analysed to test the variation between different treatments.

Results and discussion

The mean *kapas* yields obtained under different treatments applied to the two varieties and the mean yields of the two seasons are presented in tables I and II.

TABLE I

Kapas yields of American variety, 320F

Treatments	Kapas yield (Kg./ha.) 1964-65	1965-66	Mean
1. Control	894·6	1258·7	1076·65
2. Topping 6 weeks after sowing	983·1	1170·4	1076·75
3. Topping 9 weeks after sowing	1045·3	1320·9	1183·10
4. Topping 12 weeks after sowing	955·4	1260·1	1107·75
S. E.	±56·91	±57·38	
C.D.	N. S.	N.S.	

TABLE II

Kapas yields of Desi variety, Raniben

Treatments	Kapas yield (Kg./ha.) 1964-65	1965-66	Mean
1. Control	1056·3	788·5	922·40
2. Topping 6 weeks after sowing	1001·1	946·2	973·65
3. Topping 9 weeks after sowing	930·5	787·0	858·75
4. Topping 12 weeks after sowing	961·0	774·0	867·50
S. E.	±46·04	± 66·93	
C. D.	N. S.	N. S.	

It is observed from the above tables that the variations in *Kapas* yields due to different treatments were not significant. In the case of American cotton, 320F, however, there was a tendency towards increase in yield due to topping at 9 weeks after sowing. But this finding cannot be relied upon unless further confirmed through more experimentation.

Summary and conclusion

Preliminary studies for determining the effect of topping at different stages in American cotton, 320F and Desi cotton, Raniben were conducted at Government Cotton Research Station, Raya (Mathura). The results showed that topping had no significant effect on yields of the two varieties in any of the two seasons. In the case of 320F, however, topping at 9 weeks after sowing exhibited a tendency to increase the *kapas* yield. These studies need be further pursued.

Acknowledgement

The authors are grateful to the now defunct Indian Central Cotton Committee, Bombay for financing the Scheme for Agronomic and Physiological Research on Cotton in Uttar Pradesh under which these studies were carried out.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968.

Studies in Aquatic Fungi of Varanasi—V. A Taxonomic Study

By

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[Received on 21st November, 1967]

In previous communications (Dayal and Thakur, 1965 ; 1968 ; 1969*a* ; 1969*b*), we have dealt with some new and interesting species of aquatic phycomycetes centered around the University Campus during a period of over three years. Continued trapping and isolations of aquatic fungi from this region have yielded some interesting forms which have not been previously reported from this country.

Description of the Species

Achlya diffusa Harvey ex Johnson. *In* Johnson, genus *Achlya*, p. 61, 1956.

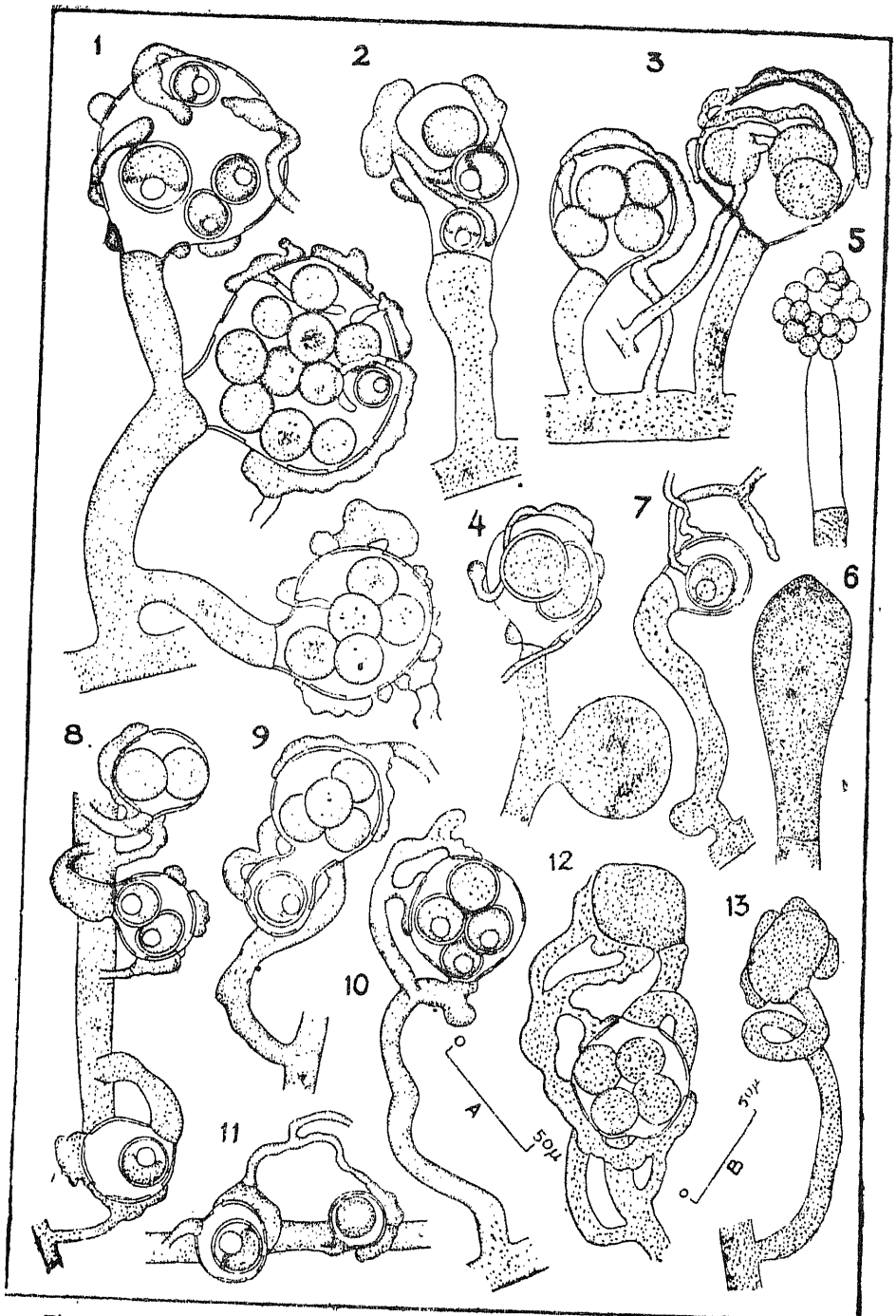
Achlya diffusa Harvey. Jour. Elisha Mitchell Sci. Soc. 58 : 29, 1942.

Mycelium moderately extensive, diffuse ; two week old colony 2-3 cm in diameter, principal hyphae slender, sparingly branched, 39-56 μ in diameter at base. Gemmae abundant, filiform, irregular, terminal or intercalary Zoosporangia abundant, filiform or clavate, 150-870 μ long by 15-44 μ in diameter predominantly 480-650 μ X 15-26 μ ; renewed sympodially. Zoospore discharge achlyoid ; spore cluster persistent at exit pore; encysted spores 10-14 μ in diameter Oogonia abundant, lateral, occasionally intercalary, 33-144 μ in diameter, predominantly 33-78 μ, immature ones freely proliferating. Oogonial wall pitting variable in frequency and size ; small oogonia unpitted or pitted under point of attachment of antheridial cells ; large oogonia pitted, oogonia like hyphal swellings intermingled with true oogonia. Antheridial branches usually present, diclinous, rarely monoclinous. Oospheres maturing, then quickly aborting ; oospores eccentric, not usually filling the oogonium, 1-11 in number, usually 3-5, 15-36 μ in diameter, predominantly 22-26 μ, germination not observed.

Isolated on maize seed from pool near Sarnath, Varanasi, U. P., India. Leg. Thakur Ji, 12 Sept., 1965. Culture RD 8. Figs. 1-4.

Achlya orion Coker and Couch. *In* Coker, Saprolegniaceae, p. 112, 1923.

Mycelium diffuse, extensive ; two week old colony 2-2·5 cm in diameter ; principal hyphae slender, 33-72 μ in diameter at base. Gemmae when present, filiform, clavate or irregular. Zoosporangia abundant, filiform or clavate, straight, occasionally curved, 60-740 μ long by 22-59 μ in diameter, predominantly 148-436 × 22-35 μ ; renewed sympodially. Zoospore discharge achlyoid, spore cluster persistent at exit pore, encysted spores 9-12 μ in diameter. Oogonia very abundant, lateral rarely terminal, spherical, 24-88 μ in diameter, predominantly 44-55 μ, immature ones infrequently proliferating. Oogonial wall smooth, always pitted under the point of attachment of antheridial cells. Oogonial stalks much



Figs. 1—4. *Achlya diffusa* Harvey ex Johnson. Fig. 1. Portion of the plant illustrating variations in number of eccentric oospores, Fig. 2. unpitted oogonium, Fig. 3. oogonia showing the extremely wide wall pits, Fig. 4. oogonium showing the characteristic hyphal swelling. Figs. 5—13. *Achlya orion* Coker and Couch. Fig. 5. Discharged zoosporangium, Fig. 6. gemma, Fig. 7. oogonium with an eccentric oospore and attendent diclinous antheridial branches, Fig. 8. portion of hypha showing three lateral oogonia, a diclinous antheridial branch, and two monoclinous ones of remote origin, Figs. 9, 10. oogonnia containing eccentric oospores positioned on a long, irregular stalk, Fig. 11. short stalk oogonia with diclinous antheridia, Fig. 12. oogonia with curved and much branched androgynous antheridium, Fig. 13. oogonium on a once coiled stalk. Figures 1—4 with scale A, rest with scale B.

larger in length, than the diameter of the oogonium stout, usually bent, curved or recurved, rarely straight or once coiled, infrequently branched. Antheridial branches usually present, androgynous, occasionally monoclinous, rarely diclinous. Oospheres maturing. Oospores eccentric, spherical, 1–14 in number, generally 1–3 ; 22-37 μ in diameter, predominantly 26-30 μ, germination not observed.

Isolated on housefly from pond water near Chiraigaon, Varanasi, U. P., India. Leg. Thakur Ji, 12 March 1966. Culture RD 17. Figs. 5–13.

Saprolegnia lapponica Gäumann. Botaniska Notiser, p. 156, 1918.

Growth delicate, two week old colony 1–1·5 cm on hempseed ; hyphae slender, straight, sparingly branched, 7-15 μ thick at base ; zoosporangia slightly greater in diameter than the hyphae, terminal, repeatedly proliferating from within ; gemmae plentiful or few, spherical, fusiform or clavate, often in moniliform chains ; oogonia generally lateral, rarely terminal borne on a short stalk or sessile, an outgrowth from the oogonial stalk often proliferating up into the oogonium, spherical, oblong, rarely, intercalary, usually cylindrical in old sporangium, 22-73 μ in diameter, wall colourless, moderately thick, pits numerous and conspicuous of unequal frequency ; antheridia did not develop in the entire culture ; oospore numerous, generally filling the entire oogonium, centric, spherical, globose or broadly ellipsoid, tawny coloured, 18–12 μ in diameter.

Isolated on hempseed from pond water behind the B. H. U. press, Varanasi, U. P., India. Leg. Thakur Ji, 16 Nov., 1966. Slide RD 24. Figs. 14–20.

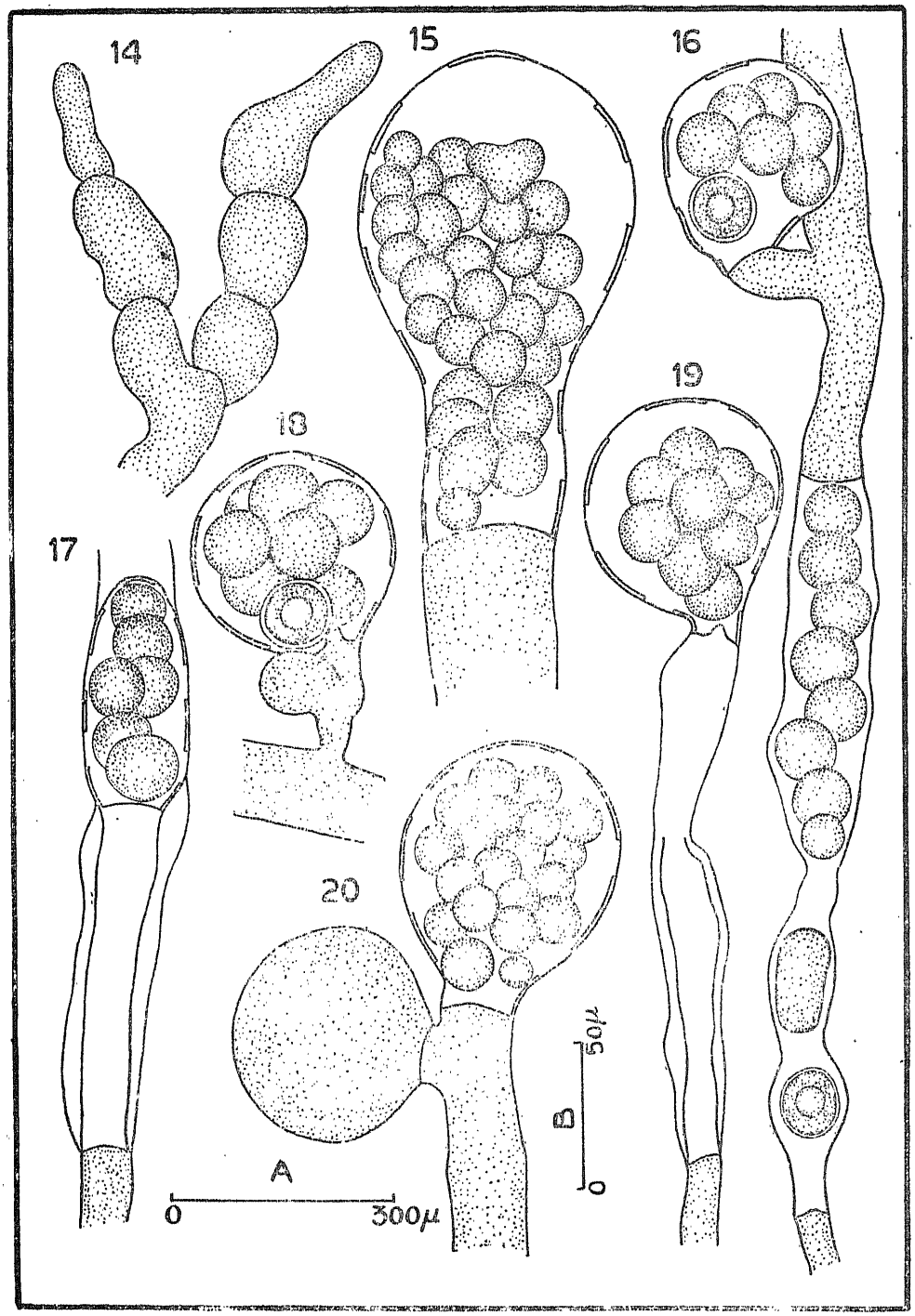
Aphanomyces irregulare Scott. *In* Scott, Genus *Aphanomyces*, p. 47, 1961.

Hyphae delicate, hyaline, sparingly branched ; zoosporangia long, filamentous, 5·5–7·4 μ in diameter, formed from undifferentiated vegetative hyphae, isodiametric ; zoospores encysting upon emergence at the orifice, 7-11 μ in diameter ; oogonia terminal on short lateral branches, spherical, 18·5–30 μ in diameter, wall roughened due to irregularities but never with definite spines or tubercles ; oospore 16–22 μ, hyaline or light brown, content finely granular with a large, central oil globule ; antheridia single, rarely two, small, clavate, antheridial stalks unbranched, short, diclinous or monoclinous ; fertilization tubes not observed ; germination of oospore by formation of a germ tube.

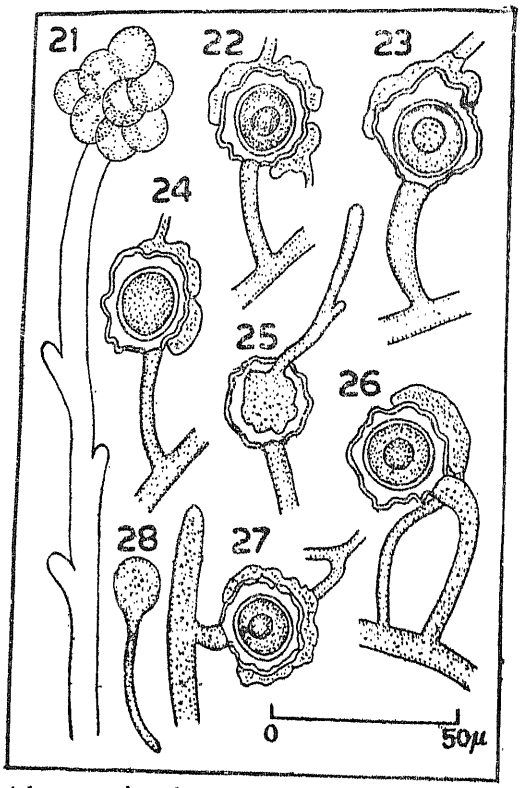
Isolated on hempseed from pond water behind B. H. U. press, Varanasi, U. P., India. Leg. Thakur Ji, 6 Nov., 1966. Slide RD 19. Figs. 21–28.

Dictyuchus carpophorus Zopf. Beiträge Z. Phys. U. Morph. n. Organismen 3 : 48, 1893.

Growth thick, two week old colony approx. 1·5 cm in diameter with main hyphae upto 85 μ thick at base. Sporangia terminal, lobed, long, clavate or slightly curved, 169–592 μ long by 19–37 μ broad, often containing a single row of spores. Oogonia terminal, single on long or short branches of the main threads, spherical, 33-56 μ in diameter, the wall pitted only under the point of antheridial attachment otherwise smooth and rarely proliferating. Antheridia always present, usually several to each oogonium, borne on slender branches of diclinous origin which often completely enwarp the oogonium. Oospore single, spherical, smooth, eccentric, 18–41 μ in diameter, predominantly 30 μ. Germination of oospore not observed.



Figs. 14—20. *Saprolegnia lapponica* Gaumann. Fig. 14. Gemmae in a chain, Fig. 15. oblong oogonium showing large number of oospheres, Fig. 16. A globose and cylindrical oogonia on the same hypha, Fig. 17. empty sporangium containing a cylindrical oogonium, Fig. 18. oogonium with conspicuous pits and with a thick walled outgrowth from below, Fig. 19. oogonium on a tip of old sporangium, Fig. 20. apical oogonium with some abnormally developed oospheres. Figure 14 drawn with scale A, rest with scale B.



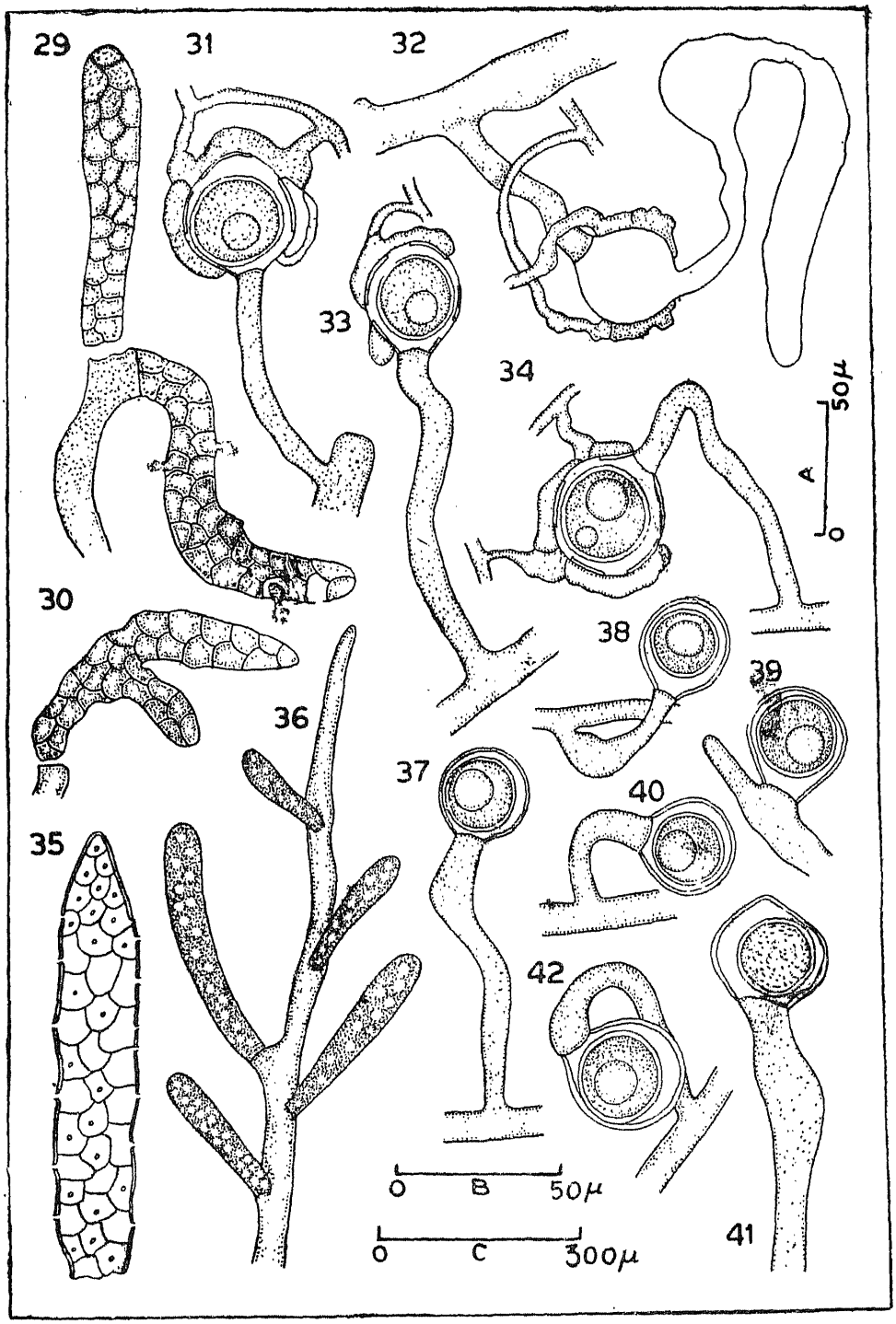
Figs. 21–28. *Aphanomyces irregulare* Scott. Fig. 21. Zoosporangium with its apical cluster of encysted zoospores, Figs. 22—24. oogonia and antheridia, Fig. 25. germinating oospore, Figs. 26—27. oogonia with monoclinous and diclinous antheridia, Fig. 28. germinating zoospore to form a mycelium.

Isolated on hempseed from pond water near Agricultural farm, Varanasi, U. P. India. Leg. Thakur Ji, 6 Nov., 1966. Slide RD 15. Figs. 29–34.

Dictyuchus missouriensis Couch. Jour. Elisha Mitchell Sci. Soc. 46 : 227, 1931.

Growth thick, broad with hyphae upto 24 μ thick, tips of hyphae rather blunt. Primary sporangia nearly cylindrical, blunt, borne typically in a zig-zag sympodium with long internodes, later they are formed by the segmentation of the hyphae into the long joints and in such case, after the spores are formed, rest like gemmae and exhibits a strong tendency to fall away from each other and from the hyphae and to lie free in the water. Sporangia terminal, long, little broad with 17–34 μ in thickness containing more than single row of spores, proliferating by repeated sprouting from the side below to form a sympodium. Spores not escaping from the sporangium as in other genera (except *Aplanes*), but remaining in the sporangium and forming there a net work of walls from which they emerge, after a rest, by individual openings to the outside.

Gemmae not represented unless resting sporangia with spores in them be considered such. Oogonia spherical, oval, smooth, the wall unpitted, terminal on long slender branches measuring about 27–34 μ in diameter, predominantly



Figs. 29—34. *Dictyuchus carpophorus* Zopf. Fig. 29. Sporangia, one detached from its hypha and other showing some zoospores have escaped through individual exit papillae while three are in the process of escaping, Fig. 30. resting sporangium of peculiar shape, Figs. 31—33. long stalked pitted oogonia with diclinous antheridia and eccentric oospore, Fig. 32. proliferating oogonium, Fig. 34. pendant oogonium with diclinous antheridia; Figs. 35—42. *Dictyuchus missouriensis* Couch. Fig. 35. Sporangium entirely empty of spores, thus leaving a 'net sporangium', Fig. 36. sporangia in resting state, Figs. 37—42. smooth walled oogonia with eccentric oospore. Figures 29—34 drawn with scale A, Figs. 35, 37—42 scale B, and Fig. 36 with scale C.

about 29 μ. Antheridia did not develop throughout the entire culture period. Oospore single, smooth, spherical, eccentric, filling almost entire inner diameter of an oogonium, 20-27 μ in diameter, predominantly 24 μ. At maturity containing only one large oil drop ranging from 10-17 μ in diameter inside the protoplasm. Germination of oospore not observed.

Isolated on hempseed from pond water near Manduadih, Varanasi, U. P., India. Leg. Thakur Ji, 20 Feb., 1964. Slide RD 60. Figs. 35-42.

Summary

During our floristic study on this little known group of fungi from Banaras Hindu University Campus, the authors had an opportunity to isolate and examine some of the unrecorded species from this country. The following species have been illustrated and described :--*Achlya diffusa*, *A. orion*, *Saprolegnia lapponica*, *Aphanomyces irregulare*, *Dictyuchus carpophorous*, and *D. missouriensis*.

Acknowledgements

We wish to express our sincerest appreciation to Professors John N. Couch, Terry W. Johnson, and Dr. Roland Seymour for their able assistance in solving problems that arose during the study. Finally the author (TJ) is grateful to the Ministry of Education, Govt. of India for the award of scholarship under which the present investigation was carried out.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968

Development of Endosperm and Embryo in *Euphorbia heterophylla* L.

By

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[Received on 18th December, 1967]

Introduction

The development of embryo sac in *Euphorbia heterophylla* L. was reported to be of the Adoxa type (Sanchez, 1938). However, reinvestigation by Maheshwari (1942) confirmed the monosporic type of embryo sac development in this species.

The present paper deals with the salient features in the development of endosperm and embryo in this species.

Endosperm

The primary endosperm nucleus divides earlier than the zygote. The nuclear division is not followed by wall formation (Fig. 1). Several free nuclei formed by repeated free nuclear divisions are arranged in the peripheral layer of the cytoplasm. Each of the nuclei as in *Euphorbia hirta* (Kajale, 1954) has 1-4 nucleoli in it. The wall formation sets in prior to the cordate stage of the embryo. It starts from the periphery and proceeds towards the centre. The cells of the endosperm, at the chalazal end are smaller in size with dense cytoplasm while those above this region are larger and vacuolate (Fig. 2) at cordate stage of the embryo. At maturity the cells of the endosperm get filled with starch. In the mature seeds a considerable part of the endosperm, surrounding the embryo, is present. Thus the seeds are endospermic.

Embryo

The fertilized egg divides only after a number of free endosperm nuclei are formed. The first division of oospore is transverse and results in terminal cell *ca* and basal cell *cb* (Fig. 3). The cell *ca* divides prior to cell *cb*. The vertical division in *ca* results in formation of two juxtaposed cells designated as *q*. The proembryo at this stage is L-shaped and consists of 3-cells, *viz.*, 2-cells derived from *ca* and 1-cell from *cb* (Fig. 4). Next division is transverse and occurs in *cb* and cells *m* and *ci* are formed (Fig. 5). The cell *ci* divides transversely to form cells *n* and *n'* (Fig. 7). The cells of the tier *q* divide transversely to form tier *l* and *l'* (Figs. 6, 7). By this time *m* undergoes vertical division (Fig. 6). The division of *m* may at times be delayed (Fig. 7). The cells in the tier *l* divide diagonally forming a group of eight cells arranged in a single tier. Four of them on the outside are designated as *a* and four in the centre as *b* (Figs. 6, 7 . The cells in *b* first divide periclinally to produce dermatogen. The derivatives of *b* differentiate into plumule while the cotyledons are derived from the derivatives of *a*. Simultaneously, with this the dermatogen differentiates in the tier *l'* as well (Fig. 8). The cells of this tier by transverse and longitudinal divisions (Fig. 8)

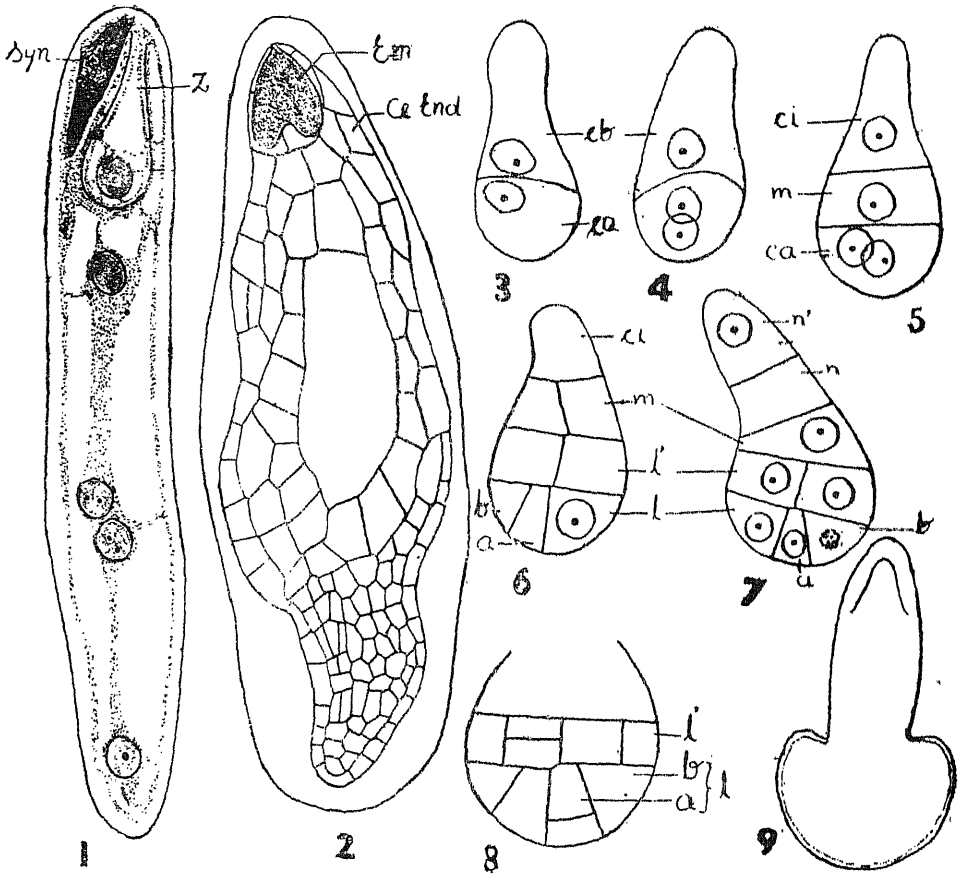


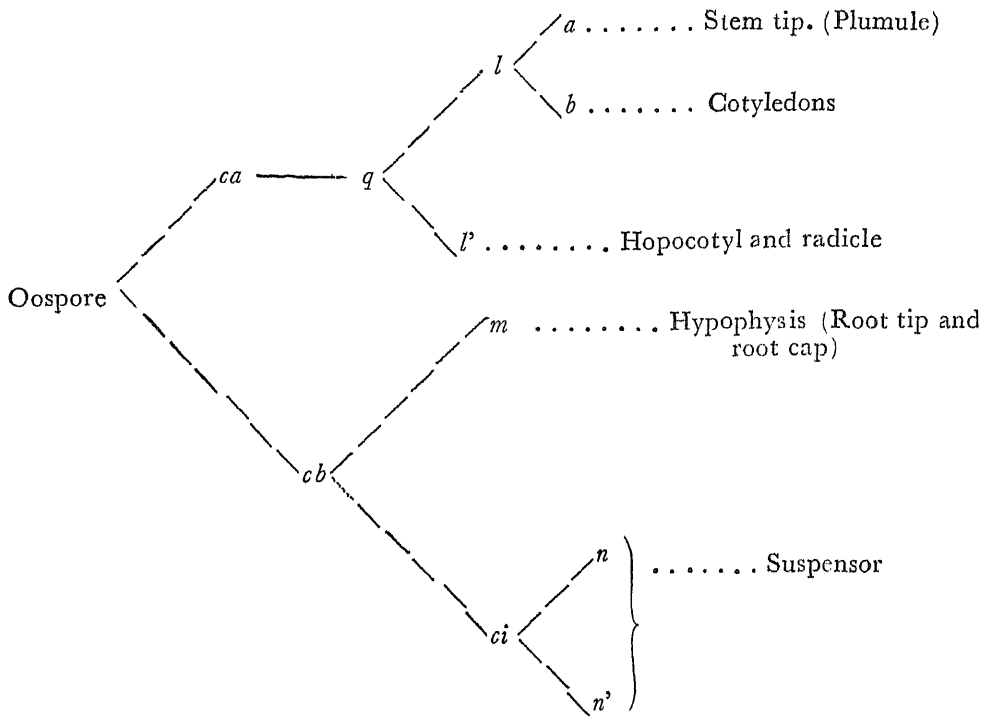
Figure 1-9. *Euphorbia heterophylla* Linn. Fig. 1. L. S. embryo sac showing zygote and 4 free endosperm nuclei. Note the degenerating synergid X 450. Fig. 2. Same as above showing cordate embryo and cellular endosperm X 200. Figs. 3-8. Stages in development of embryo X 450. Fig. 9. Mature embryo X 200.

ABBREVIATIONS

Ce.End—Cellular endosperm.
Em.—Embryo.
Syn.—Synergid.
Z.—Zygote.

form the hypocotyl and the radicle. The cell *m*, derived from basal cell *cb*, functions as hypophysis initial. As mentioned above it divides vertically first. By further divisions it gives rise to root tip and root cap. The suspensor is derived from cells *n* and *n'*.

The origin of various parts of the embryo from the oospore is given below :



Thus the embryogeny in *E. heterophylla* in all essential features resembles *E. hirta* (Kajale, 1954), *E. hypericifolia*, *E. dracunculoides*, *E. microphylla* (Mukherjee, 1957, 1961) and *Kirganelia reticulata* (Deshpande, 1959).

The mature embryo is dicotyledonous and cordate (Fig. 9).

Summary and Discussions

The nuclear type of endosperm development is characteristic of Euphorbiaceae. In *Croton sparsiflorus* and *C. klotzschianue* (*see*, Venkateshwarlu and Rao, 1963) there is formation of a free nuclear chalazal haustorium, a feature not so far reported in other members of this family. Such a feature is common in the members of the Proteaceae, the Leguminosae and the Cucurbitaceae.

The embryo development as in other members of the family (*see*, Kajale, 1954, Mukherjee, 1957, 1961, Deshpande, 1959) conforms to the Onagrad type. From the figures of Kapil (1956). Onagrad type of embryo development appears to be present in *Chrozophora obliqua* as well. *Euphorbia heterophylla*, however, differs slightly in embryo development from those of *Kirganelia reticulata* (Deshpande, 1959) and *Euphorbia microphylla* (Mukherjee, 1961). In *Kirganelia reticulata* vertical

wall first appears in *m* and thus L-shaped proembyro is not formed. Further in *K. reticulata* and *E. microphylla* both, cells *a* and *b* are not differentiated from the tier *l*.

Acknowldegments

I am thankful to Prof. L. B. Kajale for the facilities and to Dr. P. K. Deshpande for going through the manuscript and giving valuable suggestions.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968

Growth and sporulation responses of certain Fungi to mixtures of Amino acids

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[Received on 6th March, 1966]

Introduction

In nature amino acids are often available to the organisms in a mixed form. A number of investigators, therefore, have studied *in vitro* growth and sporulation responses of various fungi to mixtures of amino acids. Lilly and Barnett (1951, p. 106) observed that a combination of amino acids may or may not be better utilized than a single amino acid. The effect of an individual amino acid on the utilization of another varies with the amino acids involved and the fungus concerned. Leonian and Lilly (1940) studied the growth of *Phycomyces blakesleeanus* on five amino acids separately and in mixtures. They found better growth of the fungus in combination of mixtures than in any individual amino acid. Bilgrami (1956) carried out similar investigations on *Pestalotia mangiferae* and *Phyllosticta cycadina*. He reported that amino acids both as good and poor sources of nitrogen were simultaneously assimilated from the mixture. Tandon and Bilgrami (1957), Raizada (1957) and Ram Dayal (1959) have also observed the effects of combination of amino acids on the growth of the organisms investigated by them. This paper deals with similar studies on three pathogenic fungi, which were earlier reported by the author (Prasad, 1962) to cause leafspots of 'Litchi'.

Material and Method

Pestalotia pauciseta Sacc., *Botryodiplodia theobromae* Pat. and *Colletotrichum gloeosporioides* Penz. were isolated from diseased leaves of *Nephelium litchi* Camb. Pathogenicity of the fungi was established on the host by artificial inoculations. The effects of a number of amino acids on the growth and sporulation of these organisms were studied in previous experiments by the author (Prasad, 1963) and the dry weights were statistically analysed on the basis of which the different sources were graded as poor, moderate and good. The rates of utilization of these substances by the pathogens were also determined chromatographically by looking at the bands which disappeared with the full utilization of the substances after certain days of incubation. As the incubation period was only of 15 days, the utilization of the amino acids and their mixtures by the organisms was not studied beyond that period. The sporulation was rated as poor, fair, good and excellent depending upon the number of spores seen in the microscopic fields under the low power of the microscope as follows : 1 - 10 : poor, 11 - 20 : fair, 21 - 30 : good and 31 and above : excellent.

On the basis of the results obtained in the experiments with individual amino acids as detailed above, a combination of four amino acids consisting of both poor and good sources was selected for each of the three fungi. The KNO_3 of the basal medium (10 g glucose+3·5 g KNO_3+0·75 g $MgSO_4$, $7H_2O$+1·75 g

KH_2PO_4 mixed in a litre of distilled water) was replaced with the amino acids in such a way that the net amount of nitrogen present in the four amino acids taken together was equal to that present in 3·5 g of KNO_3. The amounts of individual amino acids in the combinations were so adjusted as to supply nitrogen in equal quantities. The pathogens were inoculated daily for fifteen days in 25 ml medium contained in Erlenmeyer flasks and the filtrates of cultures of 1 to 15 days were chromatographically analysed to study the rates of utilization of the individual amino acids from the mixtures. The procedure of Ranjan *et al.* (1955) was followed for chromatographic work. The dry weights of the fungal mats were taken after every 5 days for which experiments were run separately in triplicate. Sporulation was observed only at the end of 15 days of incubation. *P. pauciseta* was grown on a mixture of DL-phenylalanine, DL-serine, DL-lysine and L (-) cystine. Except the last one the rest had proved to be poor sources for the growth of this organism. *B. theobromae* was incubated on a mixture of DL-α-alanine, L-histidine, L (-) cystine and DL-methionine. DL-α-alanine and L (-) cystine had been found to be poor sources for this pathogen, while L-histidine a very good one. DL-methionine, however, had proved to be a very poor source. *C. gloeosporioides* was cultivated on a mixture of glycine, L-asparagin L (-) cystine and DL-methionine. Except glycine, which was found to be poor source of nitrogen for this organism, the other three amino acids served as good sources. Thus the three mixtures contained both good and poor sources of nitrogen in the form of different amino acids.

Observations

The growth data have been depicted in Figs. 1 to 3 and the utilization of the amino acids supplied individually and in mixtures as well as the sporulation gradations have been shown in the Tables 1 to 3.

TABLE 1

Utilization of amino acids (supplid individually and in mixture) by P. pauciseta *together with the effect on its sporulation*

Amino acid	Presence of amino acids in the medium — Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	Sporulation
DL-phenylalanine (supplied individually)	+ + + + + + + + + + + + – – –	Good
DL phenylalanine (supplied in mixture)	+ + + + + + + + + + + + + + +	
DL-serine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
DL-serine (supplied in mixture)	+ + + + + + + + + + + + + + +	
DL-lysine (supplied individually)	+ + + + + + + + + + + + – – –	Excellent
DL-lysine (supplied in mixture)	+ + + + + + + + + + + + + + +	
L (-) cystine (supplied individually)	+ + + + + + + + + + + + + + +	Poor
L (-) cystine (supplied in mixture)	+ + + + + + + + + + + + + + +	
Mixture	. . . . .	Fair

TABLE 2

Utilization of amino acids (supplied individually and in mixture) by B. theobromae *together with the effect on its sporulation*

Amino acid	Presence of amino acid in the medium — Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	Sporulation
Dl-α- alanine (supplied individually)	+ + + + + + + + + + + + + – –	Good
Dl-α-alanine (supplied in mixture)	+ + + + + + + + + + + + + + +	
L-histidine (supplied individually)	+ + + + + + + + + + + + + – –	Fair
L-histidine (supplied in mixture)	+ + + + + + + + + + + + + – –	
L(-) cystine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
L(-) cystine (supplied in mixture)	+ + + + + + + + + + + + + + +	
DL-methionine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
DL-methionine (supplied in mixture)	+ + + + + + + + + + + + + + +	
Mixture		Poor

TABLE 3

Utilization of amino acids (sepplied individually and in mixture) by C. gloeosporioides *together with the effect on its sporulation*

Amino acid	Presence of amino acids in the medium — Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	Sporulation
Glycine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
Glycine (supplied in mixture)	+ + + + + + + + + + + + + – –	
L-asparagin (supplied individually)	+ + + + + + + – – – – – – – –	Fair
L-asparagin (supplied in mixture)	+ + + + + + + + + + + + + + +	
L(-) cystine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
L(-) cystine (supplied in mixture)	+ + + + + + + + + + + + + + +	
DL-methionine (supplied individually)	+ + + + + + + + + + + + + + +	Fair
DL-methionine supplied in mixture)	+ + + + + + + + + + + + + – –	
Mixture		Excellent

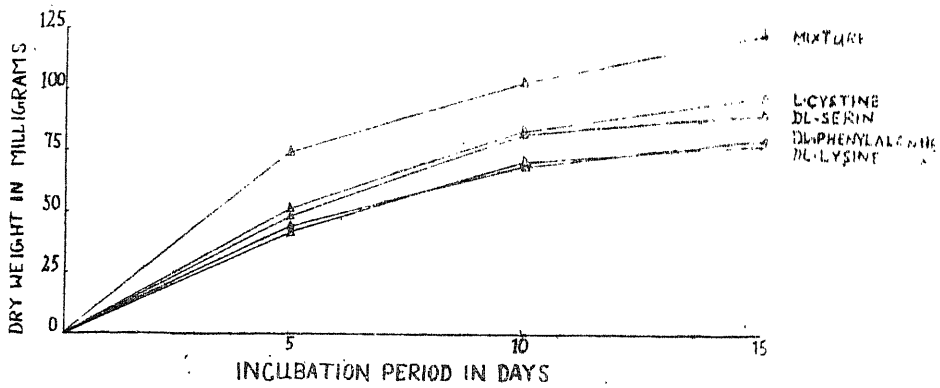

Fig. 1. Utilization of certain amino acids by *Pestalotia pauciseta* in terms of mycelial growth

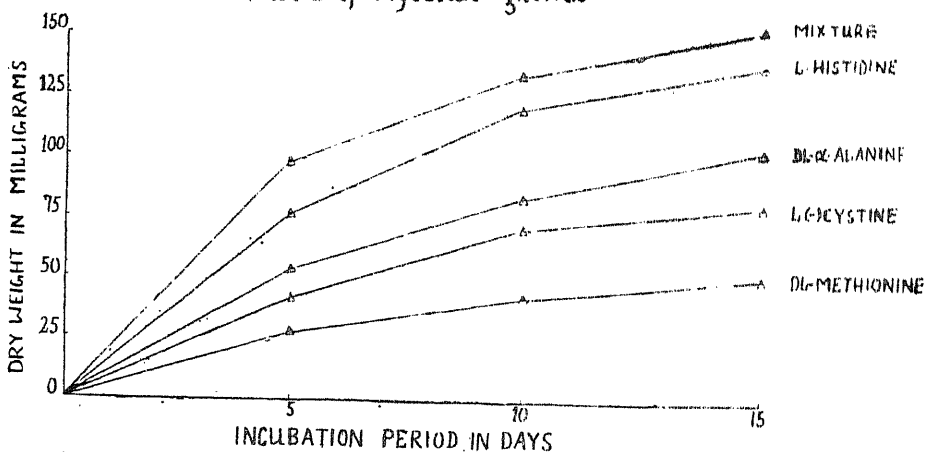

Fig. 2. Utilization of certain amino acids by *Botryodiplodia theobromae* in terms of mycelial growth

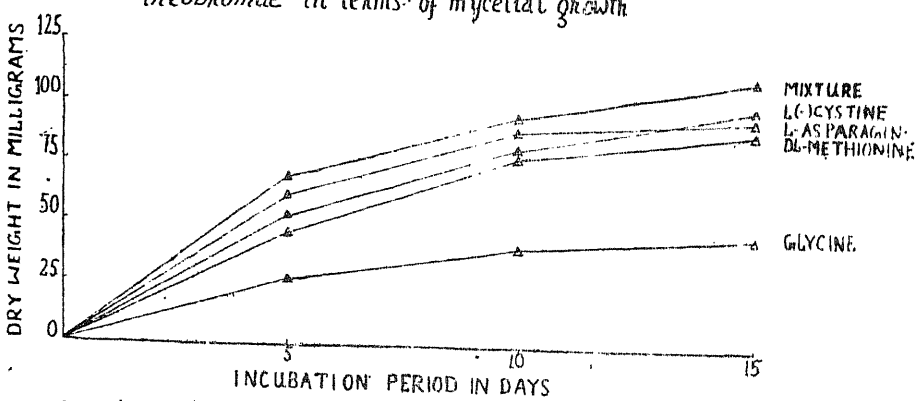

Fig. 3. Utilization of certain amino acids by *Colletotrichum gloeosporioides* in terms of mycelial growth

The results presented in the Figs. 1 to 3 showed that the total dry weights of the fungal mats of the organisms secured on the mixtures in 15 days was better than the mycelial harvest of the pathogens obtained on any individual amino acid comprising the mixtures. The rates of growth of the fungi on the mixtures showed almost the usual general pattern. It was very fast during the first 5 days, but it suddenly dropped off during the next 5 days. A steady decline in the growth rate followed during the last 5 days of incubation.

The gradual decrease in the intensity of the bands on the chromatograms indicated that the good and poor sources of amino acids were assimilated simultaneously.

It is clear from Table 1 that none of the amino acids employed could be fully utilized from the mixture by *P. pauciseta* within 15 days. Dl-phenylalanine and DL(–) lysine were used up within the incubation period when supplied individually, while Dl-serine and L(–) cystine had not been completely used up by this pathogen in 15 days. Similarly Table 2 shows that L-histidine and DL-α-alanine were consumed by *B. theobromae* within the incubation period when supplied individually but L(–) cystine and DL-methionine persisted till the end of incubation period. None of these amino acids could be fully utilized within 15 days from the mixture except L-histidine of which no trace could be found on the 14th day. Table 3 reveals that *C. gloeosporioides* had completely used up L-asparagin in 8 days only when supplied individually, while it could not consume DL-methionine, L(–) cystine and glycine within the incubation period. When supplied in mixture, glycine and DL-methionine were consumed within the incubation period, but L-asparagin and L(–) cystine persisted till the end.

From the above tables it is also clear that *P. pauciseta* produced only fair sporulation on the mixture of the amino acids. Individually DL-phenylalanine, DL-serine, DL-lysine and L(–) cystine had induced good, fair, excellent and poor sporulations, respectively. *B. theobromae* produced only poor sporulation on the mixture of the amino acids. Individually L-histidine, L(–) cystine and DL-methionine had induced fair sporulation of the pathogen, while DL-α-alanine had supported good sporulation *C. glocosporioides* developed excellent sporulation on the mixture of the amino acids. Individually all the four sources had induced only fair sporulation of this organism.

Summary and Conclusion

In each case the mixture of the amino acids proved to be a better source for the growth of the organisms than the individual amino acids. Though all the three pathogens simultaneously used the amino acids from the combinations, *B. theobromae* and *C. gloeosporioides* utilized them at different rates, but *P. pauciseta* seemed to consume both good and the poor sources at the same rate. In most of the cases the rates of utilization of the different amino acids when supplied individually had no direct relation with their utilization from the combinations and in general the suitability or unsuitability of an amino acid could not be correlated with its rate of utilization from the mixtures. Similarly the sporulations of the organisms obtained on the combinations could hardly show any relationship with the sporulation of the pathogens secured on individual amino acids. It, therefore, was evident that the same amino acids did not produce similar effects on the growth and sporulation of the present fungi when supplied individually and in mixtures.

Acknowledgement

The author is greatly indebted to Professor R. N. Tandon for guidance and his valuable suggestions.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968.

Occurrence of Bacteria Antagonistic to *Fusarium oxysporum f. cumini* and other soil fungi in cumin wilt sick-soil

By

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[Received on 6th June, 1966]

Chudiakov (1935) isolated from soil bacteria that were capable of bringing about the lysis of different species of *Fusarium* as well as other fungi. These bacteria were found to be widely distributed in most soils ; they were absent, however, in flax sick soils. Out of 34 isolates of *Bacillus subtilis* tested by Anwar (1949) against *Fusarium lini* Bolley, 10 bacterial isolates were moderately antagonistic and three inhibited the growth slightly. Naim, Mahmoud and Hussein (1957) isolated *Bacillus subtilis* which affected the morphology of *Fusarium oxysporum* Schlecht and induced antolysis and mycelial deformities. Since fungi differ in the deglree of tolerance to antagonistic bacteria, study of the bacteria isolated from cumin wilt sick soil with reference to their antagonistic effect on a number of soil fungi including the cumin wilt causing organism (*Fusarium oxysporum f. cumini* Prasad and Patel) was undertaken *in vitro* and the results are presented in this paper.

Soil from six inches diameter around the diseased plants from cumin wilt sick fields was collected in March after scraping the surface soil. Bacterial isolations were made by soil dilution plate method on potato dextrose agar. To determine the antagonistic effect, bacterial suspensions were streaked on one side of potato-dextrose-agar plate and the test fungus organism seeded on the other side. The plates were incubated at room temperature for about a week and inhibition zone measured thereafter. Pathogenic isolate of *Fusarium oxysporum f. cumini* Prasad and Patel, *Fusarium oxysporum f. corianderii* Kulkarni, Nikam and Joshi, and *F. solani* (Mart.) Sacc., *F. semitectum* Berk and Rav., *Curvularia lunata* (Wakker) Boedijn, *Sclerotium* sp., *Pythium* sp. and *Alternaria tenuis* Auct. isolated from Jaipur soils were tested in the study.

In the preliminary screening tests *Alternaria tenuis* Auct. was used as a test organism. Bacterial isolates of *Bacillus subtilis* showing antagonistic properties were selected and put to test against other fungi. The inhibition zones produced by the promising isolates of *Bacillus subtilis* as a measure of their antagonistic property against different fungi are given in table 1.

Data presented in table 1 clearly indicate that variation existed in the antagonistic property of the isolates against different fungi tested, although the most highly antagonistic isolates (B. S. 16 and 17) showed good activity against all.

TABLE

Comparative antagonistic activity of isolates of Bacillus subtilis *from cumin wilt sick soil expressed as inhibition zones in mm.*

Isolate No.	Inhibition zone in mm. Test organism							
	A	B	C	D	E	F	G	H
B. S. 2	12·5	5·0	2·5	–	3·5	5·5	–	–
B. S. 3	3·0	+	3·0	2·5	+	3·0	5·0	5·0
B. S. 4	3·5	4·5	2·0	+	–	4·5	–	–
B. S. 5	2·0	–	–	6·0	–	10·5	6·0	9·0
B. S. 6	13·0	5·0	11·0	5·0	3·0	12·0	+	8·5
B. S. 7	8·5	+	+	–	–	6·5	+	2·0
B. S. 8	12·5	3·5	7·5	3·5	4·5	11·0	+	8·0
B. S. 9	9·5	8·0	6·5	2·0	–	10·5	+	–
B. S. 10	7·0	–	–	6·5	–	5·5	4·5	11·0
B. S. 11	5·5	–	–	2·5	–	5·5	3·5	7·5
B. S. 12	11·5	2·5	4·5	6·5	–	12·5	2·5	12·0
B. S. 13	5·5	1·5	1·5	3·5	–	7·5	4·5	10·5
B. S. 14	13·5	10·0	10·0	–	–	8·5	3·0	–
B. S. 16	13·5	10·5	12·0	11·5	5·5	13·5	11·5	10·0
B. S. 17	15·0	11·0	9·5	10·0	3·5	15·0	12·0	6·5
B. S. 18	15·5	–	–	2·5	–	12·5	12·5	5·5
B. S. 19	10·5	–	–	2·5	–	7·5	+	7·5
B. S. 20	12·0	+	+	+	+	10·5	–	–
B. S. 21	11·5	–	–	4·5	–	12·5	2·5	13·5
B. S. 22	10·0	+	–	5·5	–	10·5	2·5	8·5
B. S. 23	10·5	10·0	+	5·5	–	10·0	2·5	11·0

Note:

A—*Alternari tenuis*; B—*Fusarium oxysporum f. cumini*; C—*F. oxysporum f. coriandarii*; D—*F. solani*; E—*F. semitectum*; F—*Curvularia lunata*; G—*Sclerotium* sp.; H—*Pythium* sp.
+ = Less than 1 mm.; – = Negative inhibition.

Amongst the four *Fusaria* tested, *Fusarium semitectum* Berk. and Rav. was least prone to inhibition as only 33 per cent of bacterial isolates could produce inhibition zones which too were comparatively much smaller. *Fusarium oxysporum f. cumini* and *F. oxysporum f. corianderii* showed more or less a similar trend in their susceptibility to bacterial antagonism, although marked differences were indicated with isolates B. S. 23 and 3 and variation existed in the size of the zones produced by different isolates against the two fungi. *Fusarium solani* (Mart.) Sacc. appeared to be more affected as 19 out of 21 isolates tested could induce its inhibition as compared to two vascular wilt fungi, pronounced differences existed in their reaction towards several bacterial isolates B. S. 5, 10, 11, 18, 19 and 21 producing inhibition zones, and 14 none against *F. solani* only.

All isolates exhibiting inhibitory effect against *Alternaria tenuis* were also antagonistic to *Curvularia lunata* (Wakker) Boedijn but differed considerably in the degree of inhibition incited particularly in case of isolates B. S. 2, 5 and 14. The bacteria in general showed slight effect against *Sclerotium* sp. with the exception of isolates B. S. 16 and 17 (commonly highly antagonistic) and B. S. 18 producing strong inhibitory effect. With respect to *Pythium* sp. the trend of inhibition was the same as for *Alternaria tenuis* except that four isolates (B. S. 2, 9, 14 and 20) failed to produce inhibition against this fungus and one isolate B. S. 10 was comparatively more and three isolates (B. S. 6, 17 and 18) less inhibitory.

Microscopic examination of inhibition zones in agar plates was made. Occassionally isolated hyphae grew into the intervening zone, but with this exception the growth of the fungus sooner or later was stopped. The bacteria produced a diffusable agent which diffused into agar media, producing 'stale water' that was inhibitory to the growth of the fungus. Vasudeva and Roy (1950) reported that certain strains of *Bacillus subtilis* secreted inhibitory substances and inhibited the growth of *Fusarium udum* on solid media. Vasudeva and Chakravarthi (1954) observed that the degree of inhibition varied with the different fungi, *Alternaria solani* and *Macrophomina phaseoli* being the most sensitive. At the edge of inhibition zones, various morphogenic deformities were observed which included abnormal branching and club shaped hyphal tips, heavy chlamydospore formation. The bacteria induced swelling of the germ tubes or the production of an appressorium like structure on agar (Fig. 1).

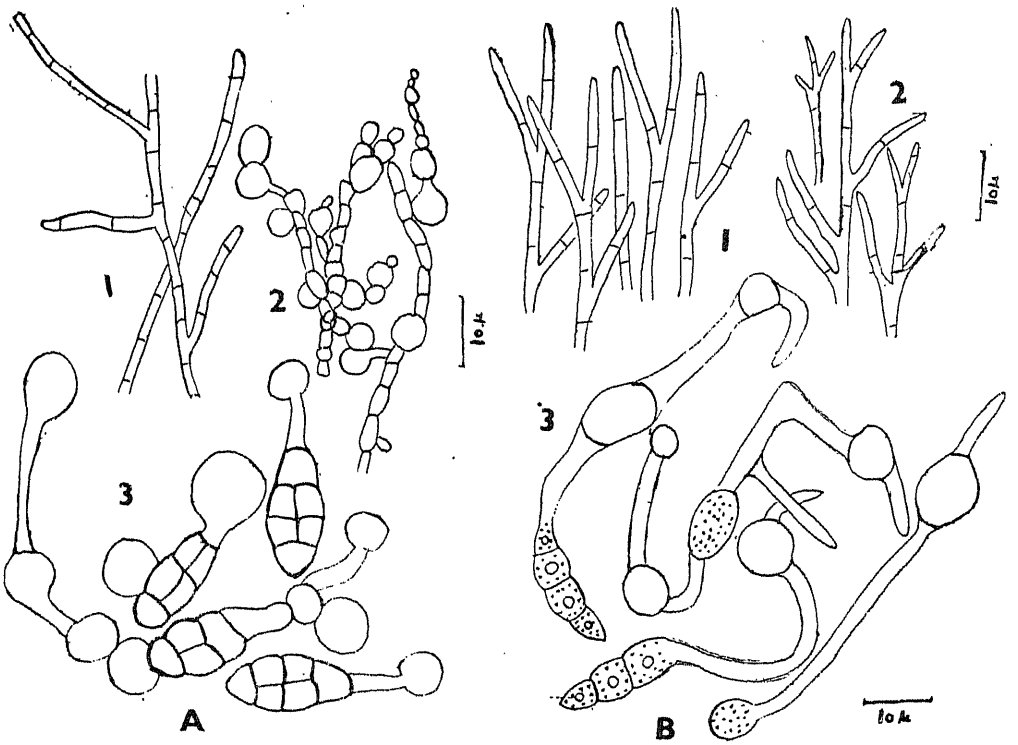

Fig. 1. Influence of *Bacillus subtilis* isolate 17 upon the growth of A—*Alternaria tenuis*; B—*Fusarium oxysporum f. cumini* 1. Normal mycelium; 2. Chlamydospore formation in *Alternaria tenuis* and abnormal branching in *F. oxysporum f. cumini*. 3. Swelling of the germ tube and production of appressorium like structure in spores.

Summary

The isolates of *Bacillus subtilis* obtained from cumin wilt sick soil have been found to inhibit the growth of cumin wilt fungus *Fusarium oxysporum f. cumini* and a number of other soil fungi. The bacterial isolates were also observed to have variability in their capacity to antagonise not only representatives of different genera of fungi but even different species and formae within the same species.

Acknowledgement

The authors express sincere thanks to the Director of Agriculture, Rajasthan, Jaipur for facilities.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968

A contribution to the embryology of Erythroxylaceae

By

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[Received on 25th January, 1968]

Introduction

Schnarf (1931) summarised the early embryological work in the family Erthroxylaceae. Later, Mauritzon (1934) studied the embryology of *Erythroxylum coca*. Narayana (1960) studied the gametophytes in *E. monogynum* and *E. mooni*; subsequently (1964) published a brief note on the embryology of *E. lanceum*. *E. cuneatum*, *E. ecarinatum*, *E. mooni* and *E. monogynum*. Most of these studies are incomplete and there is no detailed information on embryogeny, fruit and seed development. Hence the present detailed study on the embryology of *E. cuneatum* Kurz., *E. ecarinatum* Hochr., *E. kunthianum* A. St. Hil., *E. lanceum* Boj., *E. novogratanense* (Morris) Hieron., *E. novogratanense* var. *huanaca*, *E. coca* Lam. and *E. mooni* Hochr. has been undertaken in a comparative manner.

Materials and Methods

The materials were fixed in F. A. A. and then preserved in 70% ethyl alcohol. Customary methods of dehydration, infiltration and embedding were followed. The sections cut at a thickness of 7–12 microns were stained in Delafield's haematoxylin and erythrosin combination.

Observations

Microsporogenesis and male gametophyte

The male archesporium consists of two hypodermal rows of cells which show prominent nuclei and dense cytoplasm (Fig. 1). The differentiated anther shows four wall layers beneath the epidermis (Figs. 2, 4), except in *E. novogratanense* var. *huanaca* where five wall layers are formed (Fig. 3). The innermost wall layer functions as the secretory tapetum (Figs. 2–4). To start with, the tapetal cells are uninucleate with vacuolate cytoplasm (Fig. 2). When the microsporocytes enter the prophase of meiosis I, the tapetal cells become binucleate (Fig. 3) and these persist uptill the formation of uninucleate microspores (Fig. 4). The hypodermal wall layer forms the endothecium whose cells show the characteristic fibrous thickenings (Fig. 5). The middle layers and the tapetum become crushed towards the maturity of the anther (Fig. 5). There is an accumulation of tanniferous material in the epidermal cells of the anther (Figs. 2–5). The divisions of the microsporocytes follow the simultaneous method and cytokinesis takes place by peripheral cleavage furrowing (Fig. 6) resulting in tetrahedral (Fig. 7) or decussate (Fig. 8) types of microspore tetrads. Ripe pollen is 3-celled (Fig. 14). The pollen grains are 3-colpate and the exine is finely granular.

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Ovule

The ovule is crassinucellate, bitegmic and anatropous (Figs. 15–17). The ovular primordium arises as a small protuberance on the placenta and gradually grows in size. During development it bends down, then curves upwards (Figs. 15, 16) and thus becomes anatropous with an upwardly directed micropyle (Fig. 17). The funicular vascular strand terminates at the base of the chalaza (Fig. 17). The five to eight layered inner integument extends above the nucellus and forms the micropyle (Figs. 16, 17). The outer integument which is three celled thick, nearly reaches the height of the inner integument but does not take part in the formation of the micropyle (Fig. 17). The inner epidermis of the inner integument differentiates into the endothelium (Fig. 17) the cells of which are radially elongated and show prominent nuclei and vacuolate cytoplasm (Figs. 21, 22). The nucellus is elongated (Fig. 17) with 3-4 parietal layers (Figs. 19, 20).

Magasporogenesis and female gametophyte

A hypodermal archesporium is differentiated before the initiation of the integumentary primordia. It divides Fig. 18) into an upper primary parietal cell and a lower megasporocyte (Fig 15 ; the parietal cell by further divisions gives rise to a parietal tissue of three to four layers (Figs. 16, 19, 20). The megasporocyte undergoes the meiotic divisions and gives rise to a linear tetrad of megaspores (Figs. 16, 20). The lower most megaspore is functional and as a result of three sucessive free nuclear divisions gives rise to an octonucleate embryo sac. The development of the embryo sac thus follows the Polygonum type (Maheshwari, 1950). The polar nuclei fuse before fertilization and the secondary nucleus lies near the egg apparatus (Figs. 21, 22). The hooked synergids show filiform apparatus (Figs. 21, 22). The antipodals are ephemeral. During development the embryo sac elongates crushing the parietal cells above and the nucellar cells below (Figs. 17, 22). The elongation of the antipodal end of the embryo sac towards the chalazal region is particularly well pronounced and the mature embryo sac borders upon the endothelium (Fig. 22).

Fertilization

Ths entry of the pollen tube is porogamous and in *E. novogratanense* a persisting pollen tube was observed at the 2-celled stage of the embryo (Fig. 23).

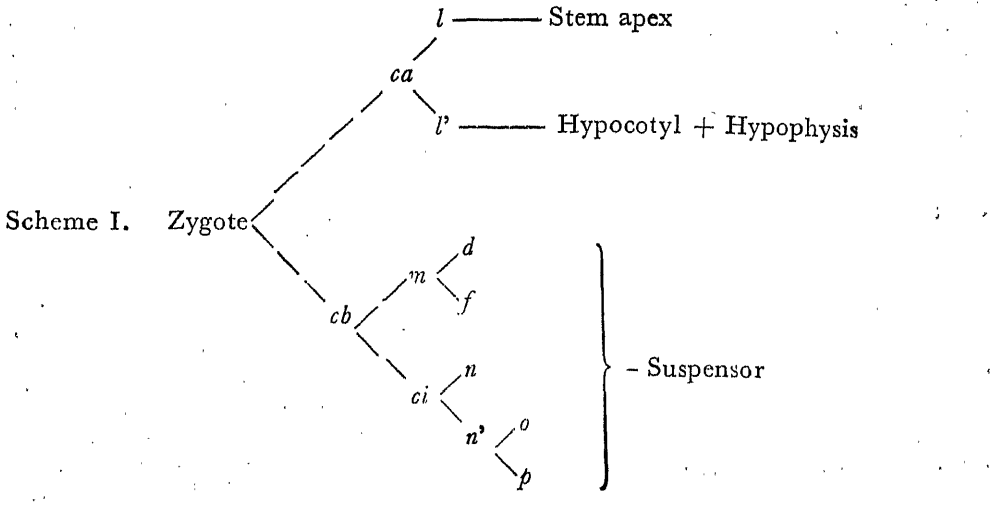
Endosperm

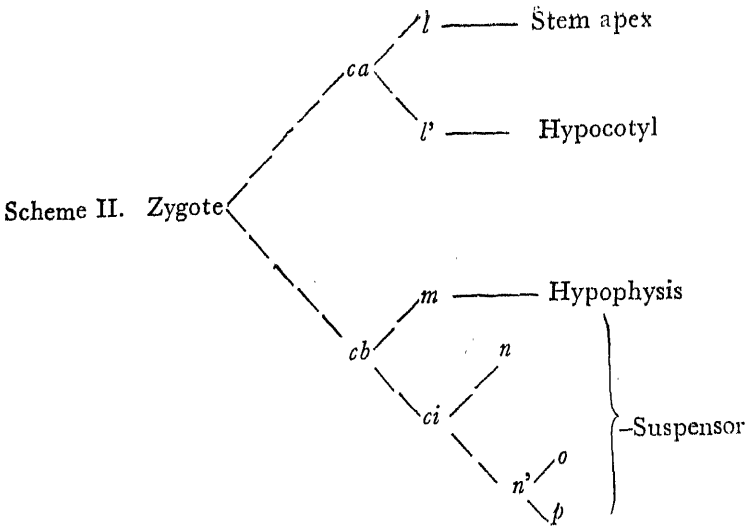
The endosperm development follows the nuclear type (Figs. 24, 25). The primary endosperm nucleus divides to form two nuclei and these divide further to form a large number of free nuclei which come to lie along the peripheral region of the elongated embryo sac (Figs. 24, 25). Usually there is an aggregation of endosperm nuclei around the developing embryo (Fig. 25). During early stages of embryo development cytokinesis proceeds centripetally along the sides and from the micropylar to the chalazal end (Fig. 25). While the globular embryo is surrounded by the endosperm tissue its lower end remains still free nuclear (Fig. 26). Ultimately this region also becomes cellular (Fig. 27). Below the cellular endosperm a few nucellar cells persist (Fig. 27). Although, a portion of the endosperm is used up by the developing embryo, it persists in the mature seed (Figs. 75, 76). The endosperm cells are thin-walled with vacuolate cytoplasm and at maturity are filled with granular materials (Fig. 28).

Embryogeny

The first division of the zygote (Figs. 29, 38, 48, 54, 57, 61) is transverse resulting in the terminal cell *ca* and the basal cell *cb* (Figs. 30, 62). Both *ca* and *cb* divide transversely forming a tetrad of four superposed cells *l*, *l'*, *m* and *ci* (Figs. 31, 40, 49, 59, 63). Sometimes, the division in *cb* precedes *ca* when the proembryo con ists of three cells arranged in a row (Figs. 39, 55, 58). Now, *l* divides vertically giving rise to two juxtaposed cells (Figs. 32, 56), while *l'* divides transversely forming two superposed cells (Figs. 32, 33, 41, 42). The cells of the tier *l* undergo another vertical division at right angles to the first resulting in quadrants (Figs. 33, 41, 42, 43, 50, 51, 64, 65). The upper daughter cell of *l'* undergoes two vertical divisions at right angles to one another (Figs. 34, 35, 42, 43), while the lower daughter cell divides giving rise to two superposed cells (Figs. 34, 35, 43, 44). Of these, upper one functions as hypohysis ; *m* also divides transversely giving rise to two superposed cells *d* and *f* (Figs. 33-35, 42-46). Sometimes the division in *m* may be delayed (Fig. 44). By about this time *n* may divide to form two superposed cells (Figs. 35, 53, 68, 69) or remain undivided (Figs. 36, 45, 46, 60). *n'* divides to form two superposed cells *o* and *p* (Figs. 35, 36, 45, 46, 65-67). Sometimes *o* and *p* further divide to form a row of four cells (Figs. 68, 69). Vertical divisions in the cells of the terminal tier *l* lead to the octant stage (Figs. 35, 44, 52). Soon the dermatogen initials are laid down in this tier and this follows the subterminal tier also (Figs. 45, 66, 67). By further divisions in the cells of the tiers *l* and *l'* the embryo becomes globular (Figs. 36, 47, 53, 60, 68). The derivatives of *l* engender the cotylendons and stem apex and those of the uppermost daughter cell of the tier *l'* give rise to the hypocotyl, while the tier below it functions as the hypophysis. In *E. coca* and *E. mooni*, the cells of *l'* undergo only vertical divisions (Figs. 50-52, 64, 65). The derivatives of *l'* engender the hypocotyl region and *m* functions as the hypophysis. The lowermost daughter cell of *l'* and the cells *d*, *f*, *n*, *o*, *p* contribute to the formation of suspensor. The embryo development thus follows the Solanad type of Johansen (1950) and keys out to the Linum-variation. The mature embryo is dicotyledonous (Fig. 37).

The following schematic representations show the derivatives of the different embryonic tiers in *Erythroxylum cuneatum*, *E. kunthianum*, *E. lanceum*, *E. novogratanense* and *E. novogratanense* var. *huanaca* (Scheme I) and *E. coca* and *E. mooni* (Scheme II)





Fruit

The furit in the various species of *Erythroxylum* is a drupe with the persistent calyx only at the base. In a young fruit, the loculus of the fertile carpel shows a single fertilized ovule and the loculi of the sterile carpels are empty (Fig. 70). However, in *E. cuneatum* rudimentary ovules were noticed. The ovary wall at this stage shows an epidermis covered by a thick cuticle. It is followed by seven to eight layers of parenchymatous cells filled with starch grains (Fig. 72) of which the hypodermal consists of palisade-like cells. The epidermis lining the loculi of the fertile and sterile carpels consists of thin walled, tangentially flattened cells with prominent nuclei and vacuolate cytoplasm (Figs. 72-74).

During post-fertilization development the pericarp undergoes certain changes and becomes differentiated into epicarp, mesocarp and endocarp. The epicarp is represented by the thickly cutinised epidermal cells with dense tanniferous contents. The mesocarp which follows the epicarp consists of a broad zone of thin-walled parenchymatous cells. The hypodermal layer and a few layers below become radially elongated and enclose numerous starch grains. The innermost layers form the endocarp. Of these, the layer lining the loculi consists of tangentially elongated, thin-walled cells with prominent nuclei and vacuolate cytoplasm. The remaining layers next to it become prominently lignified (Figs. 73, 74). The loculi of the sterile carpels become incorporated into the mature pericarp with the result there is only one seed in the fertile loculus (Fig. 71).

Seed

The seeds in *Erythroxylum* are endospermic with a straight, dicotyledonous embryo. Both the integuments contribute towards the formation of seed coats (Figs. 75, 76). At the embryo sac stage, the outer integument is three layered, while the inner is five to eight layered in thickness (Fig. 17). The cells of the epidermis of the outer integument show dense deposition of tannin (Fig. 77). The two inner layers of the outer integument are thin-walled and parenchymatous (Figs. 77, 78). To start with, the cells of the outer epidermis of the inner

integument show preminent nuclei and vacuolate cytoplasm. The inner epidermis of the inner integument which forms the endothelium is made up of radially elongated cells with prominent nuclei and vacuolate cytoplasm (Fig. 77). The cells in between increase in number and become four to seven layered (Figs. 77–80).

During seed formation the integuments undergo changes. The epidermis of the outer integument alone persists while the layers below it become pressed (Figs. 77–81). The cells of the outer epidermis of the inner integument become prominently thick-walled (Figs. 78–81) and in a longitudinal section the thicknings appear band-like (Figs. 78, 79). In *E. mooni* the outer integument at about the globular stage shows four layers and most of the cells are filled with tannin (Figs. 79). The endothelium persists as a deeply stained layer (Figs. 78, 80, 81). The thin-walled cells between the outer and inner epidermal layers become crushed and completely disappear in the mature seed coat (Fig. 81).

Discussion

The anther is 5–layered in all the invesitigated species except in *E. novogratanense* var. *huanaca*, where it is 6–layered. A fibrous endothecium is differentiated from the hypodermal layer. The binucleate anther tapetum is of the secretory type, as also reported by Narayana (1960) in *E. monogynum* and *E. mooni*. It thus corresponds to the second type of Cooper (1933). Formation of a slimy substance (Schnarf, 1931) was not noticed in any of the species investigated. The divisions of the microsporocytes follow the simultaneous type. Both tetrahedral and decussate types of microspore tetrads have been noticed in the present study. while Narayana (1960) reported only tetrahedral microspore tetrads for *E. monogynum* and *E. mooni*. According to Schnarf (1931) the pollen grains are three celled at the shedding stage. Narayana (1960) however, reported a 2–celled condition for *E. monogynum* and *E. mooni*. The present study shows that the pollen grains are three celled at the shedding stage. Unlike Linaceae the exine ornamentation is uniform. Families with such uniform ornamentation in the exine are described as stenopalynous by Erdtman (1952).

The ovule is crassinucellate, bitegmic and anatropous and an obturator is absent. It the mature ovule the outer integument is three layered and the inner is five to six layered. The micropyle is formed only by the inner integument. An endothelium consisting of radially elongated cells with prominent nuclei and dense cytoplasm is differentiated in all investigated species of this family.

The primary archesporium consists of a single hypodermal cell. A parietal tissue of three to four layers is formed. Development of embryo sac follows the Polygonum type as in other members of this family (Schnarf, 1931 ; Mauritzon, 1934 ; Narayana, 1960, 1964).

The endosperm is nuclear as reported by Narayana (1964). It becomes cellular later. About six to eight layers of this tissue persists in the mature seed.

There is no detailed account on the development of the embryo in this family. Schnarf (1931) reviewing the older literature pointed out the occurrence of a suspensor haustorium. Recently, Narayana (1964) reported that in *E. lanceum*, *E. cuneatum*, *E. ecarinatum*, *E. mooni* and *E. monogynum* embryo development conforms to the Solanad type (Johansen, 1950), but no illustrations were given. The present study on the embryogenesis of seven species of *Erythroxylum* shows that it follows the Linum-variation of the Solanad type (Johansen, 1950).

In all the species studied the fruit is a drupe. The fruit wall shows a differentiation into three parts, an outer epicarp consisting of a prominently cutinised epidermis, a middle mesocarp made up of radially elongated thin-walled

EXPLANATION TO FIGURES

Figs. 1, 19, 48–53	*Erythroxylum coca*
2, 17, 22, 58–60	*E. cuneatum*
4, 5, 12–14, 38–47	*E. kunthianum*
6–11, 15, 16, 18, 20, 24, 25, 29–37, 70–78, 80, 81	*E. lanceum*
26–28, 61–69, 79	*E. mooni*
23, 54, 57	*E. novogratanense*
3, 21, 55, 56	*E. novogratanense* var. *huanaca*

O.I. = Outer integument, I.I. = Inner integument, Ent = Endothelium, End = Endosperm, Emb = Embryo, Epi = Epicarp, Meso = Mesocarp, Endo = Endocarp

Fig. 1. T. S. anther lobe showing hypodermal archesporium.

2. T. S. anther lobe showing epidermis, three wall layers, tapetum and sporogenous cells.

3. T. S. anther lobe showing epidermis, four wall layers, the tapetum of binuclates cells and microsporocytes.

4. T. S. anther lobe showing epidermis, three wall layers, tapetum of binucleate cells and uninucleate microspores.

5. T. S. mature half anther showing epidermis, fibrous endothecium and three celled pollen grains.

6. P. M. C. showing cytokinesis.

7. Tetrahedral pollen tetrad.

8. Decussate pollen tetrad.

9. 1-nucleate pollen grain.

10. 1-nucleate pollen grain showing late anaphase.

11. Pollen grain showing lenticular generative cells and large vegetative cell.

12. 2-celled pollen grain showing the generative cell liberated into the vegetative cytoplasm.

13. 2-celled pollen grain. Note the generative cell dividing.

14. 3-celled pollen grain.

15–17. Stages in the development of the ovule.

18. L. S. portion of ovule showing the archesporial cell in metaphase.

19. L. S. portion of nucellus showing megasporocyte and four parietal cells above.

20. L. S. nucellus showing linear megaspore tetrad.

21, 22. Mature embryo sacs.

23. Micropylar part of the embryo sac showing the 2-celled proembryo and the persisting pollen tube.

24–27. Stages showing the development of endosperm.

28. Endosperm cells magnified to show prominent nuclei, vacuolate cytoplasm and granular contents.

29–36, 38–69. Stages in the development of embryo. For abbreviations see text.

37. Mature embryo.

70, 71. T. S. young and old fruits showing the development of stony endocarp.

72–74. T. S. fruit wall at different stages of development. Note the differention into outer epicarp, middle mesocarp and the inner endocarp.

75. T. S. mature seed showing cotyledons, endosperm and seed coats.

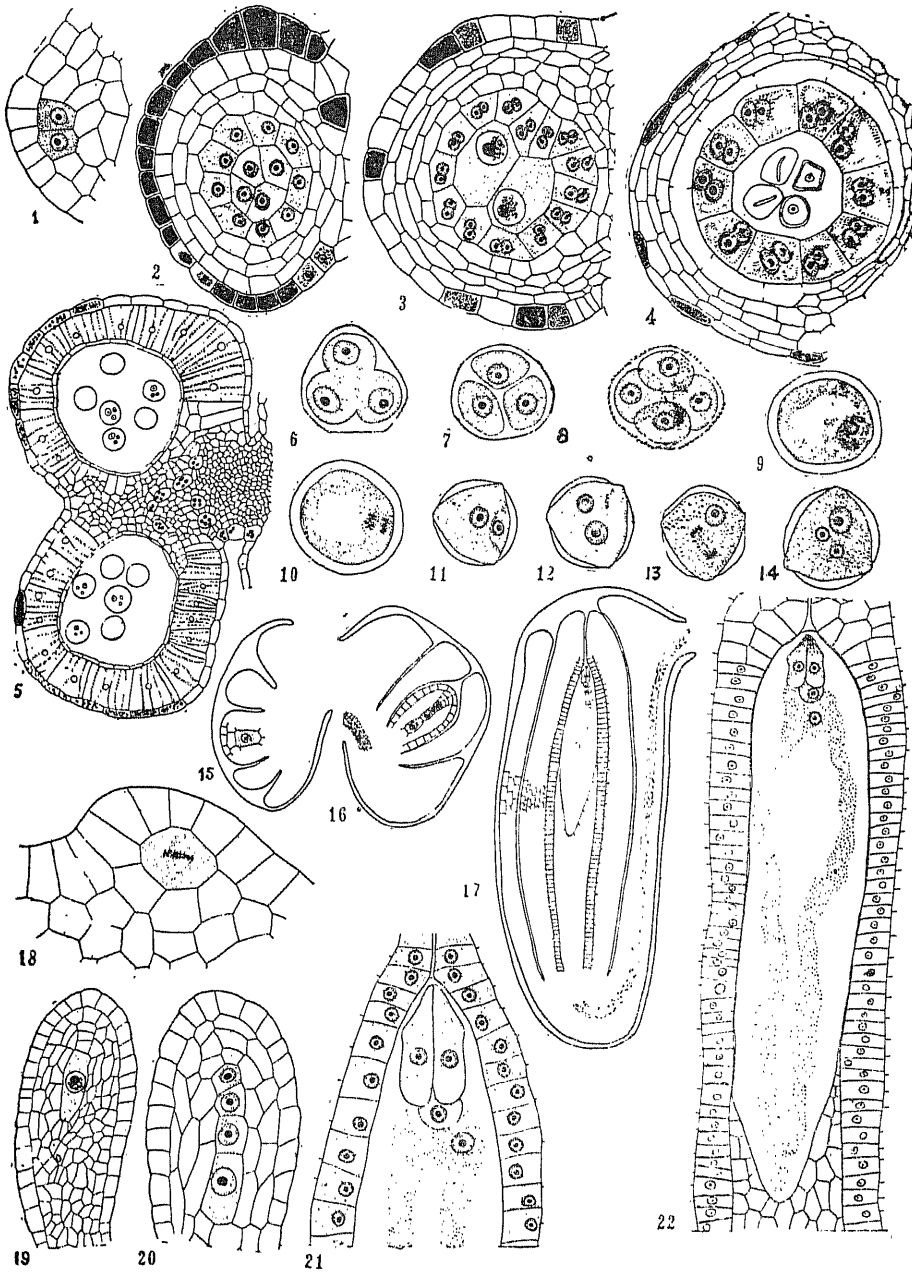
76. L. S. mature seed showing the embryo, endosperm and seed coats.

77. L. S. portion of the seed coat at the zygote stage.

78, 79. L. S. portion of the seed coat at about the globular stage of the embryo. Note the band-like thickenings in the outer epidermal cells of the inner integument.

80. T. S. portion of seed coat at the globular stage of the embryo. Note the thick-walled cells of outer epidermis of inner integument.

81. T. S. portion of mature seed coat showing testa and tegmen. The outer epidermis of the outer integument persists; the cells inner to it become pressed. The tegmen is formed by the outer and inner epidermal layers of the inner integument.



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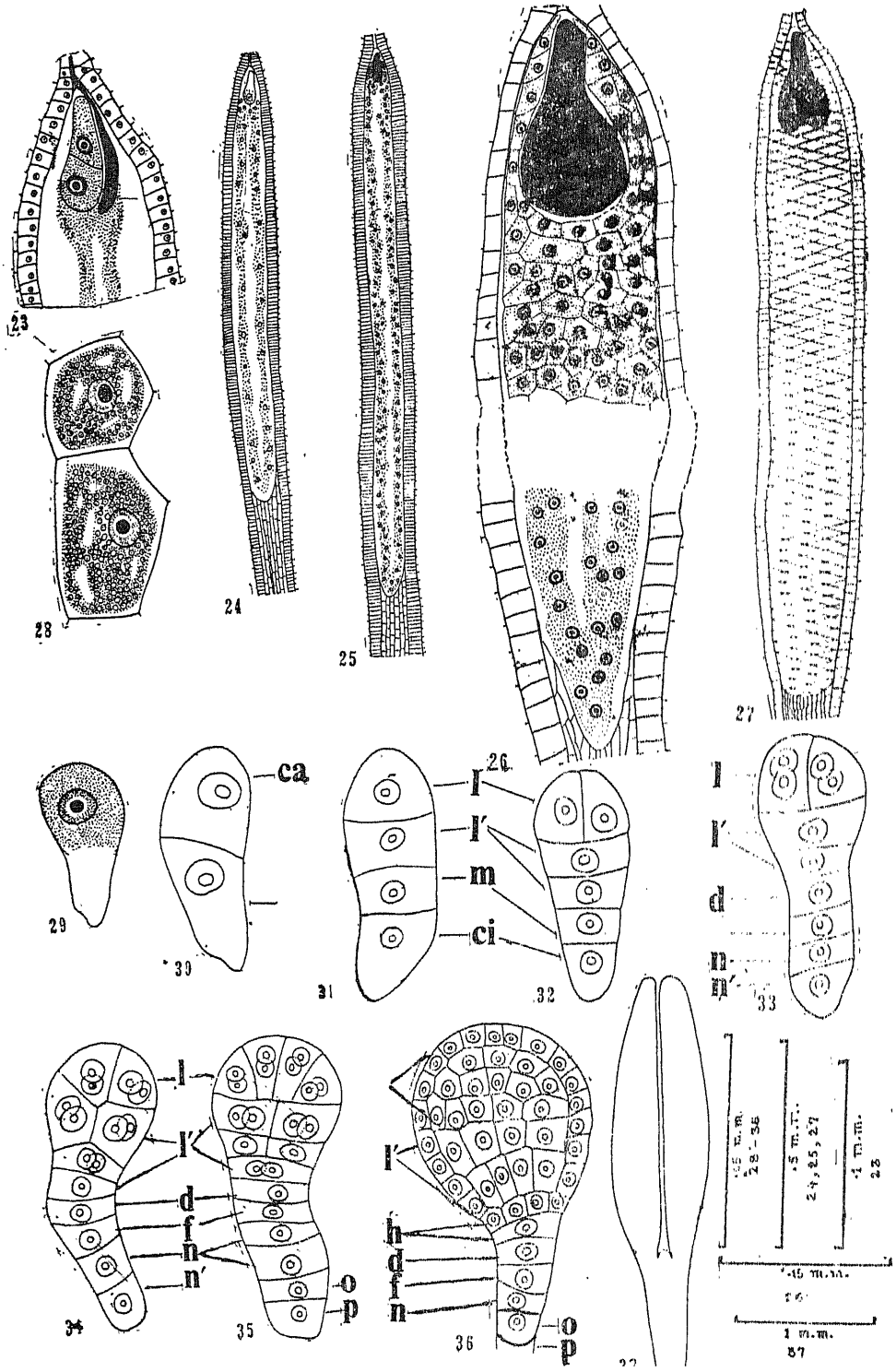
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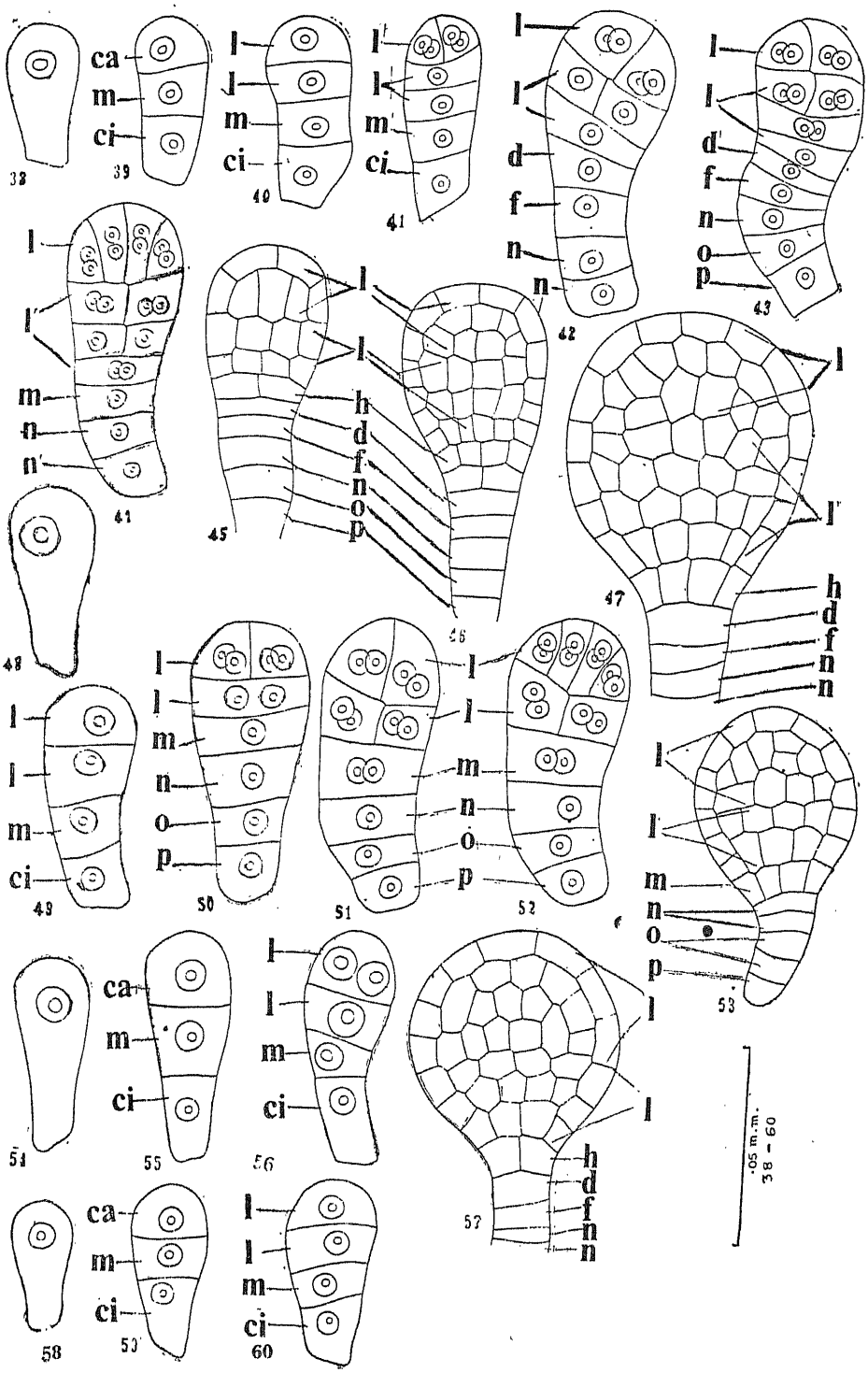
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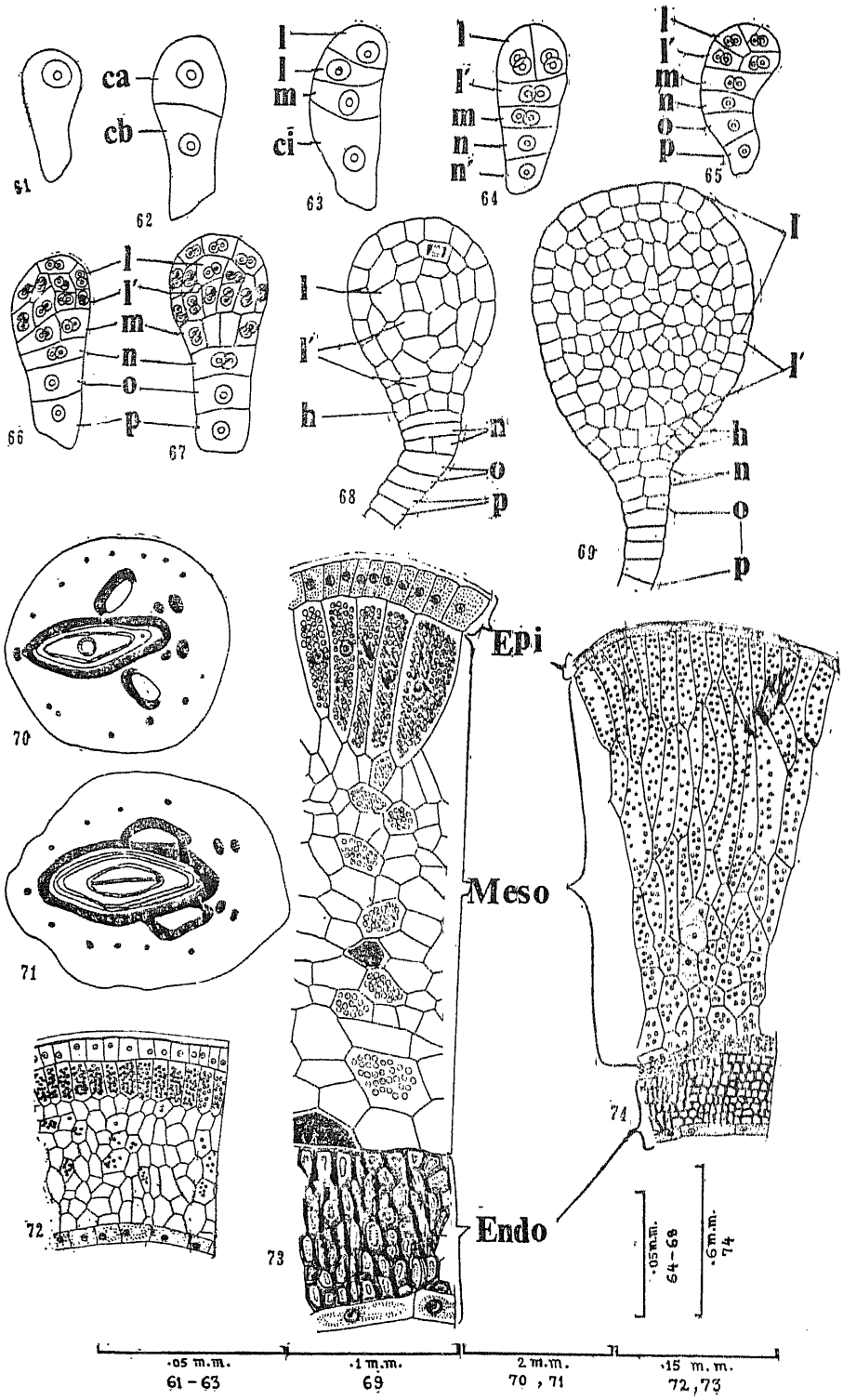
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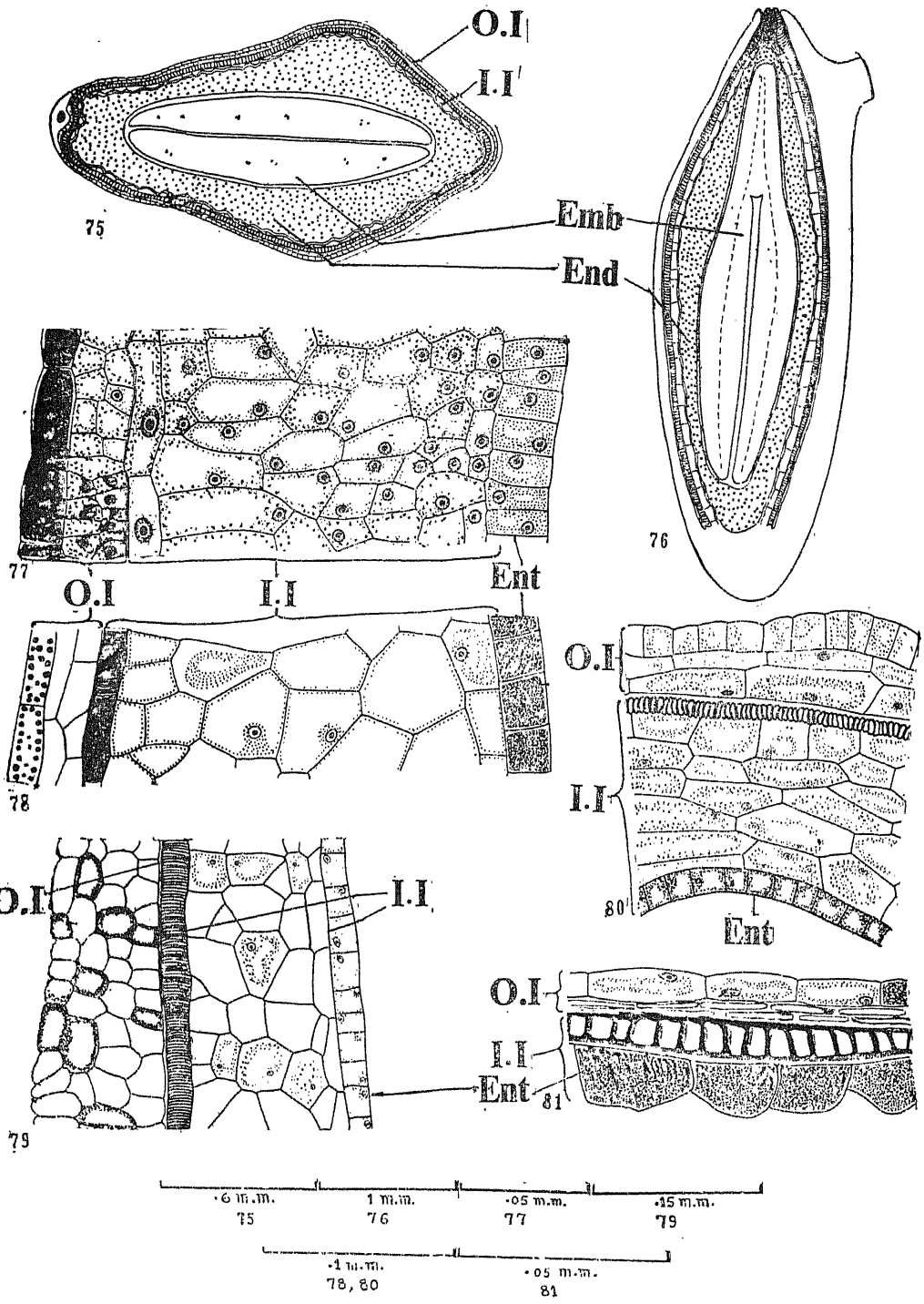
·05 m.m.
61 - 63
·1 m.m.
69
2 m.m.
70 , 71
·15 m. m.
72,73
·05m.m.
64-69
·6m.m.
74


O.I
I.I
75
Emb
End
76
77
O.I
I.I
Ent
78
O.I
I.I
80
Ent
O.I
I.I
Ent
81
79
·6 m.m.
75
1 m.m.
76
·05 m.m.
77
·15 m.m.
79
·1 m.m.
78, 80
·05 m.m.
81

parenchymatous cells stored with starch grains and an inner endocarp represented by prominently lignified cells. At maturity the sterile carpels become incorporated into the fruit wall.

Both the integuments take part in the formation of the seed coats. At maturity, the outer epidermis of the outer integument persists while the two inner layers become pressed. The cells of the outer epidermis of the inner integument become thick-walled and the inner epidermis differentiates into the endothelium which persists in the seed. The cells in between are crushed. The seed is endospermic in all the species.

The Erythroxylaceae resemble the Linaceae in the following embryological characters ; structure and development of the anther and the male gametophyte ; crassinucellate, bitegmic, anatropous ovules ; single celled archesporium ; Polygonum type of embryo sac ; early fusion of polar nuclei ; ephemeral nature of antipodals, nuclear endosperm ; Solanad type of embryogeny and endospermic seeds.

In the uniformity of exine ornamentation and absence of obturator the Erythroxylaceae stand apart from members of Linaceae. While the similarities in embryological features indicate a possible relationship between these families, the differences justify their separation into independent families a conclusion supported by findings from floral anatomy (Rao, 1956) and wood anatomy (Heimsch, 1942).

Summary

Embryology of *Erythroxylum ceneatum*, *E. ecarinatum*, *E. kunthianum*, *E. lanceum*, *E. novogratanense*, *E. novogratanense* var. *huanaca*, *E. coca* and *E. mooni* has been studied in a comparative manner.

The primary archesporium in the anther consists of two hypodermal rows of cells. The differentiated anther shows four to five wall layers beneath the epidermis. The hypodermal wall layer develops into the endothecium with characteristic fibrous thickenings ; while the innermost forms the secretory tapetum of binucleate cells. Tannin is abundant in the epidermal cells of the anther. The microsporocytes divide in a simultaneous manner ; cytokinesis takes place by peripheral cleavage furrowing and forms tetrahedral or decussate pollen tetrads. The tricolpate pollen grains are shed at 3-celled stage.

The ovule is crassinucellate, bitegmic and anatropous with an upwardly pointed micropyle which is formed by the inner integument. An endothelium is differentiated from the innermost layer of the inner integument. The nucellus is elongated. The funicular vascular strand terminates at the base of chalaza.

The hypodermal archesporium in the ovule is single celled. The parietal cell forms three to four parietal layers. The megaspore tetrad is linear. The embryo sac development conforms to the polygonum type. The synergids are hooked and show filiform apparatus. The polar nuclei fuse before fertilization. Antipodals are ephemeral. Entry of the pollen tube is porogamous.

Endosperm is nuclear. Cytokinesis between endosperm nuclei proceeds in a centripetal manner from the micropylar to the chalazal end. At maturity the cells of the endosperm show granular materials.

Development of the embryo conforms to the Solanad type and keys out to the Linum-variation.

The fruit is a drupe with persistent calyx at base. The loculi of the sterile carpels become incorporated in the fruit wall at maturity. The pericarp at

maturity differentiates into epicarp, mesocarp and endocarp. The seeds are endospermic. The seed coats are formed by both the integuments. In the outer integument only the outer epidermis persists and the two inner layers become crushed. In the inner integument the thick-walled outer epidermis and the endothelium persist while the middle layers are completely crushed.

Acknowledgements

I take this opportunity to express my grateful thanks to Dr. L. L. Narayana for suggesting the problem, guidance and encouragement throughout the progress of the work. My sincere thanks are due to Prof. M. R. Suxena, for his kindly interest and encouragement. I am indebted to Dr. Anwari Dilmy for the materials of *E. lanceum*, *E. coca*, *E. cuneatum*, *E. ecarinatum*, *E. novogratanense* and *E. novogratanense* var. *huanaca* ; to Dr. A. S. Rao for *E. kunthianum* and to the Superintendent, Royal Botanic Gardens, Peradeniya for the material of *E. mooni*. Thanks are also due to the C. S. I. R. for the award of a Junior Research Fellowship.

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Proc. Nat. Acad. Sci., India, 38(B), I, 1968

Ecology of soil fungi of cultivated fields*

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[Received on 2nd May, 1967]

Numerous workers, *viz.* Chaudhary and Sachar (1934), Ghatak and Roy (1939), Jasevoli (1924), Jensen (1931), and Piston (1930) studied the soil fungal flora of cultivated fields in India and abroad. A few researchers compared the fungal population of cultivated fields with those of natural soils (Bisby *et al.* 1933, Dixon 1928, Janke and Holzer 1929). Garrett's (1950, 1951, 1952), researches opened a new era in the study of soil fungi by correlating the distribution of fungal population with the ecological factors. Later on, the studies on ecology of soil fungi were carried on in different parts of the world by workers like, Warcup (1951), Saksena (1955), Tresner *et al.* (1954), Orput and Curtis (1957) Dwivedi (1960), Saxena and Sarbhoy (1962) and Mishra (1964).

However, in India, the ecology of the soil fungi of cultivated fields was not attempted properly and the fragmentary information available was far from complete. It was considered, therefore, worth while to investigate the ecology of soil fungal population of cultivated fields of *Cicer arietinum* Linn. and *Lens esculenta* Moench. Meth. the two most important leguminous crops of India.

Materials and Methods

Four plots, each of *Lens esculenta* and *Cicer arietinum*, were selected. Plots I and II in the case of *Lens* were outside the Banaras Hindu University campus and III and IV were inside. In the case of *Cicer*, all the plots were situated in the University campus itself. Except for the plots I and II of *Lens*, others plot were occasionally irrigated.

The soil samples were collected from the upper 6″ of the soil and the fungal population was assessed by inoculating the soil solution of 1:100 and 1:1000 dilutions. The conventional dilution-plate method was followed, using Waksman's medium of the following composition :

Dextrose, 10 g.; peptone, 5 g.; KH_2PO_4, 1 g.; $MgSO_4$, $7H_2O$, 0·5 g.; agar, 20 g.; distilled water, 1000 cc., Rose Bengal 1:15,000

The fungal population was recorded after 5-6 days of incubation at 25°C.

Soil samples collected were analysed for determining the moisture content, pH, water-holding capacity, organic matter, nitrate, exchangeable Ca, Mg and phosphate, M.C., W.H.C., exchangeable Ca, Mg and phosphate were determined by the methods suggested by Piper (1949) ; pH was determined by Beckman's electric pH meter and nitrate by colorimetric method of Snell and Snell 1948. Organic carbon was estimated by phenolamine method and organic matter by multiplying the organic content by the arbitrary factor, 1·724.

The records of the fungal population of the various localities are tabulated in the Tables I—II. The data on soil analysis are given in the Tables III—X.

*Part of a thesis approved for the award of Ph.D. degree of Banaras Hindu University

TABLE I

Distribution of Fungal Species in different plots of Lens esculenta

Fungal species	PLOTS			
	I	II	III	IV
Mucor luteus Linn.	+	+	+	+
M. hiemalis Wehmer	−	+	+	+
Gongronella butleri (Lendn.) Peyronel and Dal Vesco	−	+	−	+
Rhizopus nigricans Ehrenberg	+	+	+	+
R. oryzae Went and Gerlings	−	−	−	+
Phycomycetous sterile colony	+	−	+	+
Pythium debaryanum Hesse	−	−	−	+
Zygorhynchus heterogamus (Vuill).	−	+	+	+
Choanephora cucurbitarum (Berkley and Ravenel) Thaxtar	+	+	+	+
C. circinans Nagarishi and Kawakimi	−	+	−	−
Cunninghamella blakesleeana Lendner	−	−	+	+
Syncephalastrum racemosum (Cohn) Schroeter	−	+	−	−
Thielavia terricola (Gilman and Abbott) Emmons.	+	+	+	+
Chaetomium terrestre Dwivedi	−	−	−	+
C. globosum Kunze	+	+	+	+
Melanospora sp.	−	−	−	+
Neocosmospora vasinfecta E. F. Smith	+	+	+	+
Chaetoceratostoma longirostre Farrow	−	−	+	+
Aspergillus nidulans (Eidam) Wint	+	+	+	+
A. montevidensis Talice and Mackinnon	+	+	+	−
Penicillium spiculisporium Lehman	+	+	+	+
P. brefeldianum Dodge	+	−	+	+
Phoma hibernica Grimes, O'Connor and Cummins	+	+	+	+
P. humicola Gilman and Abbott	+	+	−	−
Macrophoma sp.	+	−	−	−
Botryodiplodia sp.	+	+	−	−
Chaetomella raphigera M. F. Swift	−	−	+	+
Trichoderma viride Pers. ex Fr.	+	+	+	+
T. glaucum Abbott	+	−	−	−
Aspergillus niger Van Tiegham	+	+	+	+
A. terreus Thom	+	+	+	+
A. flavus Link	+	+	+	+
A. candidus Link	+	+	+	+
A. sydowi (Bain. and Sar.) Thom & Church	+	+	+	+
A. sulphureus (Fres.) Thom and Church	+	+	+	+
A. japonicus Saito	+	+	+	+
A. fumigatus Fres.	+	+	+	+
Penicillium humicola Oud.	+	+	+	+
P. notatum Westling	+	+	+	+

Table I (*contd.*)

Fungal species	PLOTS I	II	III	IV
P. funiculosum Thom	+	+	+	+
P. purpurogenum Stoll	+	+	+	+
P. variabile Sopp.	−	−	−	+
Paecilomyces fusisporus Saks.	+	+	+	+
P. varitoti Bainier	+	+	+	+
Acremonium sp.	+	−	−	−
Cephalosporium asperum Marchal	+	+	+	+
Acrotheca sp.	−	−	−	+
Gliocladium fimbriatum Gilman & Abbot	−	−	−	+
Oedocephalum coprophilum Tubaki	−	−	−	+
Curvularia lunata (Walker) Boedijn	+	+	+	+
C. geniculata (Tracy & Earle) Boedijn	−	−	+	+
C. tuberculata Jain	+	+	−	−
Cladosporium herbarum (Pers.) Link	+	+	+	+
Alternaria tenuis Nees.	+	+	+	+
A. humicola Oud.	−	+	+	+
Papulospora sp.	+	+	+	+
Humicola fusco-atra Traaen	+	+	+	+
Scolecobasidium terreum Abbott		−	+	+
S. constrictum Abbott	+	−	−	−
Helminthosporium anomalum Gilman & Abbott	+	+	+	+
Hormiscium sp.	+	+	−	+
Nigrospora sphaerica (Sacc.) Mason	+	+	−	−
Fumago sp.	+	+	−	+
Periconia sp.	+	−	−	−
Teracocosporeum sp.	+	−	−	−
Phaeotrichoconis sp.	−	−	−	+
Memnoniella echinata (Rivotta) Galloway	−	+	−	+
Stachybotrys aurantia Baron	−	+	+	−
Fusarium nivale (Fries) Cesati	+	+	+	+
F. oxysporum Schlechtendahl	+	+	+	+
F. tricinctum (Cda.) Emend	+	+	+	+
F. roseum (LK.) Emend	+	+	+	+
F. chlamydosporum Wollenweber & Reinking	+	+	−	−
Myrothecium varrucaria (Albertini & Schweinitz Ditmar	+	+	+	+
Epicocum nigrum Link	−	+	−	+
Pestalotia monorhinca Speg.	−	−	+	+
Sclerotium sp.	+	+	+	+
Sterile colony	+	+	+	+
	54	53	51	63

TABLE II

Distribution of Fungal Species in Cicer arietinum *Plots*

Fungal species	PLOTS I	II	III	IV
Mucor luteus Linn	+	+	+	+
M. hiemalis Wehmer	+	+	+	+
M. plumbeus Bonorden	+	+	+	+
Gongronella butleri (Lendn.) Peyronel and Dal Vesco	+	+	+	+
Rhizopus nigricans Ehrenberg	+	+	+	+
R. oryzae Went and Gerlings	−	+	−	+
Sterile phycomycetous colony	+	+	+	+
Zygorhynchus heterogamus (Vnill.)	+	+	+	+
Choanephora cucurbitarum (Berkley & Ravenel) Thaxter	+	+	+	+
Cunninghamella blakesleeana Lendner	+	+	+	+
Syncephalastrum racemosum (Cohn) Schroeter	−	+	+	+
Pythium debaryanum Hesse	+	−	−	−
Thielavia terricola (Gilman & Abbott)	+	+	+	+
Chaetomium globosum Kunze	+	+	+	+
C. terrestre Dwivedi	−	+	+	−
C. funicola Cooke	−	−	+	+
Neocosmospora vasinfecta E. F. Smith	+	+	+	+
Melanospora sp.	−	−	+	−
Choetoceratostoma longirostre Farrow	−	+	−	−
Aspergillus nidulans (Eidam) Wint.	+	+	+	+
Penicillium spiculisporium Lehman	−	−	+	+
P. brefeldianum Dodge	−	+	+	−
Phoma hibernica Grimes, O'Connor & Cummins	+	+	+	+
Chaetomella raphigera M. E. Swift	+	−	+	+
Robillarda sessilis Sacc.	−	−	−	+
Botryodiplodia sp.	+	−	−	−
Trichoderma viride Pers. ex Fr.	+	+	+	+
Aspergillus niger Van Tiegham	+	+	+	+
A. terreus Thom	+	+	+	+
A. flavus Link	+	+	+	+
A. candidus Link	+	+	+	+
A. Sydowi (Bain. and Sar.) Thom & Church	+	+	+	+
A. sulphureus (Fres.) Thom and Church	−	+	+	+
A. japonicus Saito	+	−	−	−
A. awamori Nekjawa	−	+	−	−
A. fumigatus Fres.	−	−	+	−
Penicillium humicola Oud.	+	+	+	+
P. notatum Westling	+	+	+	+
P. funiculosum Thom.	+	+	+	+
P. purpurogenum Stoll	+	+	+	+
P. rubrum Stoll	+	+	−	−

Table II (*contd.*)

Fungal species	PLOTS I	II	III	IV
P. restrictum Gilman & Abbott	+	−	−	−
P. variabile Sopp.	+	−	+	+
Paecilomyces fusisporus Saks.	+	+	+	+
P. varioti Bainier	−	−	−	+
Fusidium sp.	−	+	−	−
Acremonium sp.	−	−	+	−
Cephalosporium coremioides Raillo	+	−	+	+
C. acremonium Corda	+	−	−	−
Monilia geophila Oud.	−	−	+	−
Gliocladium roseum (Link.) Thom.	+	+	−	+
G. fimbriatum Gilman & Abbott	−	−	−	+
Curvularia lunata (Walker) Boedijn	+	+	+	+
C. geniculata (Tracy and Earle) Boedijn	+	−	+	+
C. pallescens Boedijn	+	−	−	−
Cladosporium herbarum (Pers.) Link.	+	+	+	+
Alternaria tenuis Nees.	+	+	+	+
A. humicola Oud.	+	+	+	+
Papulospora sp.	+	+	+	+
Humicola fusco-atra Traaen	+	+	+	+
Stigmella sp.	−	−	−	+
Scolecobasidium terreum Abbott	+	+	+	+
S. constrictum Abbott	−	+	+	−
Helminthosporium anomalum Gilman & Abbott	+	+	+	+
H. flagelloideum Atkinson	−	−	−	+
Hormiscium sp.	+	−	−	−
Fumago sp.	−	−	−	+
Nigrospora sphaerica (Sacc.) Mason.	+	−	+	+
Phaeotrichoconis sp.	−	+	−	−
Periconia sp.	−	−	+	+
Stachybotrys aurantia Baron	+	+	−	−
Memnoniella echinata (Rivolta) Galloway	−	−	+	−
Tetracoccosporium sp.	−	+	−	−
Fusarium nivale (Fries) Cesati	+	−	+	+
F. oxysporum Schlechtendahl	+	+	+	+
F. tricinctum (Cda.) Emend.	+	+	+	+
F. roseum (LK.) Emend.	+	+	+	+
F. chlamydosporum Wollenweber & Reinking	−	−	−	+
Myrothecium verrucaria (Albertini & Schweinitz) Ditmar	+	+	+	+
M. roridum Tode	−	+	+	+
Epicoccum nigrum Link.	+	+	−	−
Pestalotia monorhinca Speg.	−	+	+	−
Sclerotia rolfsii Sacc.	+	+	+	+
Sterile colony	+	+	+	+
	56	56	60	60

TABLE III

Data on analysis of soil samples of Lens *plot I*

Date	Moisture content %	pH	Water holding capacity %	Organic matter %	NO_3/ 100 g. of soil	Exch. cal. M. E. %	Phos-phate parts/ million	Exch. Mg. M.E. %
25 Nov. 1963	7·33	6·85	43·73	1·21	9·65	24·32	0·99	1·43
10 Dec. 1963	9·90	6·90	42·51	1·09	10·00	25·42	0·85	1·43
25 Dec. 1963	9·80	7·00	44·63	1·15	10·23	25·00	0·89	1·43
10 Jan. 1964	6·30	7·10	45·51	1·11	10·05	24·69	1·00	1·52
25 Jan. 1964	12·20	7·20	44·63	1·20	9·90	24·23	1·01	1·45
10 Feb. 1964	11·20	6·90	42·79	1·18	11·32	24·80	0·98	1·52
25 Feb. 1964	5·42	6·86	44·83	1·20	11·01	23·43	1·02	1·42
10 Mar. 1964	6·69	6·80	44·63	1·20	10·60	22·65	1·01	1·53
25 Mar. 1964	3·80	6·90	41·72	1·21	10·35	24·63	1·20	1·42
15 Nov. 1964	7·60	6·85	41·25	1·22	11·65	23·24	0·98	1·54
30 Nov. 1964	3·30	6·80	44·52	1·20	11·09	23·32	0·98	1·43
15 Dec. 1964	4·20	6·90	45·32	1·22	10·01	24·69	0·91	1·43
30 Dec. 1964	5·00	7·01	39·83	1·30	10·65	24·42	0·90	1·23
15 Jan. 1965	3·80	7·10	36·97	1·21	10·13	24·42	0·90	1·23
30 Jan. 1965	3·40	7·00	41·42	1·21	10·13	24·53	1·02	1·42
15 Feb. 1965	2·80	6·90	43·45	1·26	11·65	22·65	1·01	1·32
28 Feb. 1965	2·80	6·85	42·13	1·31	11·90	23·01	0·96	1·45

TABLE IV

Data on analysis of soil samples of Lens *plot II*

Date	Moisture content %	pH	Water-holding capacity	Organic matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M.E. %
25 Nov. 1963	6·12	6·70	42·18	1·19	9·00	24·23	0·92	1·4
10 Dec 1963	9·90	7·00	41·73	1·16	8·90	23·41	0·85	1·32
25 Dec. 1963	9·80	7·10	40·19	1·11	9·20	22·62	1·20	1·48
10 Jan. 1964	10·90	7·20	41·12	1·12	10·30	23·12	1·10	1·68
25 Jan. 1964	13·12	6·90	42·69	1·11	9·60	24·63	0·98	1·53
10 Feb. 1964	9·00	6·80	42·13	1·21	9·89	23·13	0·89	1·43
25 Feb. 1964	7·00	6·80	43·13	1·10	10·65	21·62	0·86	1·49
10 Mar. 1964	4·50	6·90	44·62	1·10	10·90	24·42	0·90	1·57
15 Nov. 1964	6·04	7·00	45·47	1·21	10·65	23·52	1·01	1·68
30 Nov. 1964	4·30	7·10	45·12	1·20	10·26	22·73	0·93	1·52
15 Dec. 1964	4·70	6·85	33·50	1·11	11·36	21·42	0·86	1·54
30 Dec. 1964	2·50	6·80	44·13	1·11	11·56	24·63	1·00	1·63
15 Jan. 1965	4·05	6·90	44·60	1·10	10·65	22·68	0·96	1·62
30 Jan. 1965	3·10	7·00	43·32	1·12	10·65	24·27	0·86	1·63
15 Feb. 1965	2·30	7·10	45·32	1·10	9·85	23·42	1·08	1·59
28 Feb. 1965	2·00	6·90	44·41	1·01	9·65	24·51	0·95	1·47

TABLE V

Data on analysis of soil samples of Lens *Plot III*

Date	Moisture content	pH	Water holding capacity %	Organic matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
25 Nov. 1963	15·80	6·75	39·02	1·21	10·63	21·04	1·75	0·93
10 Dec. 1963	14·60	6·70	40·61	1·20	11·42	22·12	1·71	0·91
25 Dec. 1963	9·90	6·95	41·73	1·20	11·63	22·12	1·83	0·95
10 Jan. 1964	10·00	7·10	42·09	1·21	10·41	20·31	1·87	0·93
25 Jan. 1964	9·50	7·00	42·75	1·20	10·63	21·19	1·62	0·98
10 Feb. 1964	5·80	6·95	41·29	1·11	11·09	20·05	1·67	1·02
25 Feb. 1964	4·60	7·00	40·18	1·11	10·43	20·36	1·60	1·04
10 Mar. 1964	2·30	7·10	40·63	1·11	10·62	22·85	1·58	1·02
25 Mar. 1964	1·20	7·00	42·63	1·12	11·43	20·96	1·47	1·02
15 Nov. 1964	7·20	6·92	40·84	1·21	10·63	19·21	1·68	0·93
30 Nov. 1964	9·75	6·90	41·09	1·22	11·08	19·36	1·63	0·98
15 Dec. 1964	10·50	6·80	42·05	1·20	11·64	19·05	1·65	0·94
30 Dec. 1964	5·60	6·85	41·53	1·21	11·08	20·05	1·64	0·99
15 Jan. 1965	4·40	6·95	39·64	1·21	12·09	20·90	1·58	1·03
30 Jan. 1965	4·60	6·90	38·69	1·20	11·62	21·03	1·57	0·99
15 Feb. 1965	3·40	7·10	38·81	1·22	11·82	20·63	1·68	0·96
28 Feb. 1965	3·10	7·10	39·21	1·21	11·09	21·85	1·63	1·06
15 Mar. 1965	4·20	7·00	39·53	1·21	11·63	21·65	1·63	1·05
30 Mar. 1965	11·80	7·00	39·63	1·22	11·84	21·34	1·65	0·90

TABLE VI

Data on analysis of soil samples Lens *Plot IV*

Date	Moisture content %	pH	Water holding capacity %	Organic matter	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
25 Nov. 1963	16·80	6·70	40·58	1·31	8·23	18·65	1·38	0·93
10 Dec. 1963	16·00	6·65	40·62	1·29	9·42	19·03	1·47	0·94
25 Dec. 1963	11·90	7·10	40·52	1·21	10·17	20·65	1·03	1·03
10 Jan. 1964	6·66	7·10	40·08	1·21	9·99	21·90	0·98	1·02
25 Jan. 1964	9·00	6·90	41·83	1·20	10·41	20·80	0·96	0·93
10 Feb. 1964	9·40	6·95	40·18	1·21	10·43	21·80	0·96	0·94
25 Feb. 1964	1·00	7·10	40·15	1·11	10·53	20·56	1·33	0·96
10 Mar. 1964	2·00	7·10	40·41	1·10	10·63	21·46	1·52	0·98
25 Mar. 1964	1·00	7·10	40·15	1·11	10·53	20·56	1·33	0·96
15 Nov. 1964	10·70	7·00	39·78	1·52	9·43	20·56	1·42	1·03
30 Nov. 1964	11·20	6·90	39·13	1·43	9·82	21·65	1·00	1·02
15 Dec. 1964	11·90	6·90	40·77	1·40	10·44	20·90	1·01	1·04
30 Dec. 1964	12·00	6·85	44·32	1·39	10·32	21·90	0·99	0·94
15 Jan. 1965	12·00	6·90	42·77	1·21	10·83	20·65	0·99	0·97
30 Jan. 1965	8·20	7·00	42·19	1·21	10·34	20·15	1·00	0·96
15 Feb. 1965	8·60	6·90	41·83	1·20	10·27	20·60	1·03	1·11
28 Feb. 1965	4·30	7·00	40·73	1·21	10·43	20·89	1·11	1·02
15 Mar. 1965	3·20	7·10	43·21	1·14	10·82	20·66	1·37	1·06
30 Mar. 1965	7·70	7·10	42·18	1·13	10·81	21·03	1·00	1·01

TABLE VII

Data on analysis of soil samples of Gram plot I

Date	Maisture content %	pH	Water holding capacity %	Organic matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
1 Nov. 1963	12·50	6·85	39·62	1·65	9·65	16·43	1·90	0·95
15 Nov. 1963	9·95	6·95	40·03	1·52	10·65	18·32	1·87	0·99
1 Dec. 1963	9·17	7·10	41·73	1·49	10·65	17·42	2·05	1·04
15 Dec. 1963	8·40	7·20	41·51	1·49	10·05	18·24	2·05	0·98
1 Jan. 1964	20·90	6·90	39·45	1·35	9·08	18·27	1·96	0·95
15 Jan. 1964	11·00	7·00	30·15	1·21	10·80	19·43	1·87	0·90
1 Feb. 1964	8·00	7·10	41·63	1·20	9·65	19·42	1·87	0·85
15 Feb. 1964	6·30	7·20	40·51	1·15	10·23	19·64	1·93	0·25
1 Mar. 1964	5·60	6·90	39·68	1·29	9·65	20·43	1·90	0·82
15 Mar. 1964	2·00	6·90	39·73	1·25	10·85	20·24	2·00	0·70
10 Nov. 1964	7·90	7·00	32·99	1·32	9·65	20·43	1·86	0·89
25 Nov. 1964	8·80	7·20	41·57	1·02	9·80	19·62	1·67	0·86
10 Dec. 1964	8·60	7·10	41·77	1·65	9·80	19·62	1·67	0·86
25 Dec. 1964	10·00	7·00	43·62	1·46	9·90	18·42	1·98	0·93
10 Jan. 1965	10·90	6·90	44·07	1·39	8·90	18·42	1·65	0·80
25 Jan. 1965	5·80	6·85	33·63	1·51	9·01	18·42	2·01	0·90
10 Mar. 1965	1·60	6·75	40·53	1·47	9·98	19·30	1·89	0·95
25 Mar. 1965	7·40	6·85	39·51	1·58	10·70	18·31	1·63	1·05

TABLE VIII

Data on analysis of soil samples of Gram plot II

Date	Moisture content %	pH	Water holding capacity %	Organic matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
1 Nov. 1963	13·28	6·90	40·63	1·99	3·65	18·25	1·00	0·98
15 Nov. 1963	11·32	6·90	39·92	1·78	8·30	19·42	1·09	0·85
1 Dec. 1963	6·50	7·00	38·55	1·72	7·40	20·63	0·98	0·83
15 Dec. 1963	5·40	7·20	42·13	1·58	9·30	19·42	1·20	1·03
1 Jan. 1964	16·20	7·00	41·63	1·46	10·60	18·25	1·09	0·96
15 Jan. 1964	13·70	7·10	40·51	1·56	11·80	20·43	1·05	0·81
1 Feb. 1964	11·40	6·85	42·18	1·48	10·80	20·63	1·07	0·62
15 Feb. 1964	8·30	6·90	42·63	1·51	11·23	20·27	1·01	0·87
1 Mar. 1964	6·00	6·90	39·63	1·50	9·65	21·00	1·01	0·87
15 Mar. 1964	1·09	7·00	39·13	1·43	7·68	20·68	1·08	0·86
10 Nov. 1964	5·70	6·75	41·52	1·96	7·90	19·53	1·08	1·03
25 Nov. 1964	9·07	7·00	32·50	1·89	8·00	18·42	1·05	1·00
10 Dec. 1964	8·80	7·10	39·17	1·77	8·65	19·43	1·08	0·81
25 Dec. 1964	11·50	7·10	40·42	1·76	10·60	18·24	1·02	0·83
10 Jan. 1965	12·50	6·80	39·53	1·67	9·65	19·21	1·07	0·83
25 Jan. 1965	10·00	7·00	40·17	1·64	10·65	19·34	2·01	0·91
10 Mar. 1965	1·50	6·85	39·21	1·49	10·65	20·65	1·06	0·91
25 Mar. 1965	8·80	6·90	40·52	1·49	10·65	20·63	1·09	0·91

TABLE IX

Data on analysis of soil samples of Gram plot III

Date	Moisture content %	pH	Water holding capacity %	Organic Matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
1 Nov. 1963	15·4	6·85	43·18	1·98	12·82	19·90	1·08	1·73
15 Nov. 1963	7·4	6·95	44·19	1·98	11·73	19·65	0·97	1·62
1 Dec. 1963	10·2	7·00	42·83	1·91	10·62	20·65	0·93	1·72
15 Dec. 1963	6·8	7·00	39·72	1·78	12·42	20 90	1·03	1·62
1 Jan. 1964	5·3	6·90	39·93	1·89	10·32	20·80	1·06	1·82
15 Jan. 1964	7·2	6·90	41·52	1·77	11·43	20·80	0·98	1·82
1 Feb. 1964	4·8	7·15	40·63	1·73	10·80	20·80	0·98	1·83
15 Feb. 1964	2·9	7·20	41·83	1·70	10·63	19·80	1·02	1·73
1 Mar. 1964	7·25	7·15	42·52	1·67	11·48	18·96	1·02	1·73
10 Nov. 1964	6·9	6·95	44·44	1·99	12·32	20·45	0·83	1·51
25 Nov. 1964	4·1	7·15	42·13	1·97	11·61	20·36	0·89	1·43
10 Dec. 1964	3·2	7·20	38·89	1·97	12·42	21·65	0·91	1·62
25 Dec. 1964	9·5	7·15	38·19	1·95	12·62	18·50	0·97	1·52
10 Jan. 1965	11·1	7·00	35·65	1·94	11·67	19·65	0·93	1·42
25 Jan. 1965	9·5	7·15	38·19	1·95	12·62	18·50	0·97	1·52
10 Feb. 1965	7·6	7·10	37·86	1·81	12·07	20·32	1·03	1·57
25 Feb. 1965	10·1	7·00	36·52	1·88	12·04	21·65	1·02	1·62
10 Mar.1965	4·1	7·15	37·86	1·69	11·62	19·65	0·82	1·53
25 Mar.1965	9·9	7·00	38·92	1·98	12·09	20·65	0·95	1·54

TABLE X

Data on analysis of soil samples of Gram plot IV

Date	Maisture content %	pH	Water holding capacity %	Organic matter %	NO_3/ 100 g. of soil	Exch. Ca. M. E. %	Phos-phate parts/ million	Exch. Mg. M. E. %
1 Nov. 1963	14·2	7·10	38·62	2·11	10·63	19·65	1·47	0·95
15 Nov. 1963	8·0	6·95	39·01	1·98	11·42	19·03	1·72	0·94
1 Dec. 1963	7·3	6·85	38·93	1·90	11·72	20·65	1·49	1·02
15 Dec. 1963	5·4	7·00	36·62	1·79	11·82	20·90	1·62	1·03
1 Jan. 1964	5·6	6·85	38·41	1·68	10·40	20·80	1·64	1·01
15 Jan. 1964	6·1	6·90	40·06	1·56	10·42	20·65	1·63	0·98
1 Feb. 1964	4·7	7·05	41·93	1·57	10·25	19·65	1·78	0·93
15 Feb. 1964	2·5	7·10	40·45	1·48	11·62	19·69	1·63	0·89
1 Mar.1964	1·1	7·15	40·42	1·48	10·43	19·95	1·59	0·59
10 Nov. 1964	6·04	7·00	38·86	2·10	11·79	19·65	1·73	0·94
25 Nov. 1964	3·04	7·10	40·25	2·10	11·62	19·60	1·52	0·94
10 Dec. 1964	3·80	7·00	38·43	1·98	10·43	20·15	1·73	1·02
25 Dec. 1964	5·80	6·85	40·10	1·98	9·68	21·65	1·63	1·01
10 Jan. 1965	6·60	6·95	38·57	1·85	9·47	20·85	1·62	1·06
25 Jan. 1965	13·40	6·90	39·63	1·85	10·46	21·65	1·79	1·02
10 Feb. 1965	5·40	6·95	39·37	1·76	9·27	20·85	1·73	1·73
25 Feb. 1965	10·40	7·00	40·23	1·47	9·43	19·90	1·52	0·82
10 Mar.1965	4·10	7·10	37·41	1·54	10·62	21·85	1·48	0·93
25 Mar.1965	7·1	6·95	39·21	1·48	9·75	20·90	1·59	0·98

Result and Discussion of Data

Distribution of the fungi in the *Lens* plots.—Four, Seven and Forty-three species of Phycomycetes, Ascomycetes and Deuteromycetes were obtained from plot I. All the Phycomycetes were isolated infrequently. Except *Thielavia terricola*, other Ascomycetes isolated were of infrequent occurrence. *Aspergillus niger, A. terreus, Paecilomyces fusisporus, Curvularia lunata, Cladosporium herbarum* and *Fusarium tricinctum* were the dominant deuteromycetous forms.

Plot II harboured 8, 6 and 39 species of Phycomycetes Ascomycetes and Deuteromycetes respectively. Except *Rhizopus nigricans* which was sometimes present with high frequency other forms were of rare occurrence. Ascomycetous forms obtained from the plot were of infrequent occurrence. The dominent forms of Deuteromycetes were almost the same as in plot 1.

Plot III harboured, 7, 8 and 36 species of Phycomycetes, Ascomycetes and Deuteromycetes respectively. *Mucor luteus, Rhizopus nigricans* and a phycomycetous sterile colony were with high frequency, whereas others were isolated infrequently. Of the Ascomycetes, only *Thielavia terricola* was of high frequency and others were of occasional occurrence. *Trichoderma viride, Aspergillus niger, A. flavus, Penicillium humicola, Paecilomyces fusisporus. Curvularia lunata, Cladosporium herbarum,* and *Fusarium tricinctum* were the dominant forms amongst the Deuteromycetes.

Plot IV harboured the highest number of species, *i.e.* 63 ; 10, Phycomycetes; 9, Ascomycetes and 44, Deuteromycetes. *Mucor luteus, Rhizopus nigricans* and phycomycetous sterile colony were obtained with high frequency. Amongst Ascomycetes. *Thielavia terricola* showed high frequency, whereas others were of occasional occurrence. The dominent deuteromycetous species were *Aspergillus niger. A. terreus, A. flavus, Penicillium humicola, Paecilomyces fusisporus. Curvularia lunata, Alternaria humicola, Cladosporium herbarum* and *Fusarium nivale* (Table I).

Ten, four and forty-two species of Phycomycetes, Ascomycetes and Deuteromycetes were isolated from plot I of *Cicer. Mucor hiemalis, Rhizopus nigricans, Choanephora cucurbitarum* were the only phycomycetous forms with high frequency, and others were with a low frequency. *Thielavia terricola, Chaetomium globosum, Neocosmospora vasinfecta* and *Aspergillus nidulans* were the ascomycetous forms isolated occasionally. Amongst Deuteromycetes, *Aspergillus niger, A. terreus, A. flavus, Penicillium humicola, Paecilomyces fusisporus, Curvularia lunata, Cladosporium herbarum* and *Fusarium nivale* were the dominant ones.

Plot II harboured 11, 7 and 38 species of Phycomycetes, Ascomycetes and Deuteromycetes respectively. Amongst Phycomycetes, *Rhizopus nigricans* was the only dominant form. Ascomycetes forms were isolated infrequently with a low frequency. The dominant species of Deuteromycetes were *Aspergillus niger, A. flavus, A. terreus, Penicillium humicola, Paecilomyces fusisporus, Curvularia lunata, Cladosporium herbarum* and *Fusarium nivale.*

Sixty species were isolated from plot III of which 10, 8 and 42 were from Phycomycetes, Ascomycetes and Deuteromycetes respectively. *Rhizopus nigricans* was the only dominant form amongst the Phycomycetes. Among Ascomycetes, *Thielavia terricola* was the only dominant form, whereas others were represented by very low frequency. *Penicillium humicola, P. notatum, Paecilomyces fusisporus, Curvularia lunata, Cladosporium herbarum, Alternaria tenuis* and *Fusarium tricinctum* were the dominant forms of Deuteromycetes.

Plot IV also possessed the same number of fungal species as that of plot III. Amongst the Phycomycetes the dominant species were *Mucor hiemalis, Rhizopus*

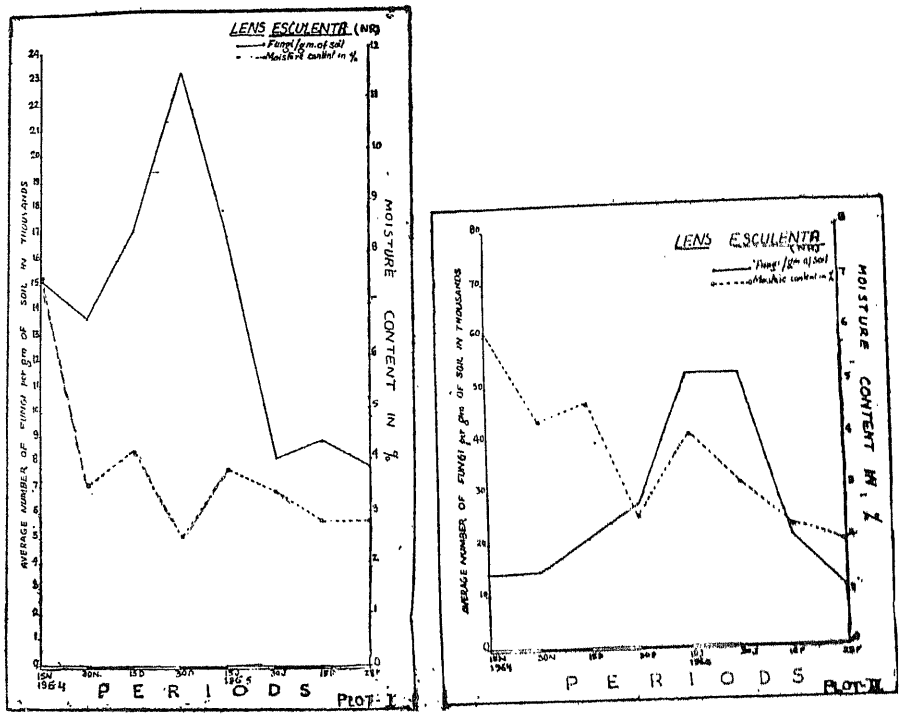


Plate I. Effect of moisture content on the non-rhizosphere fungal population of *Lens esculenta* (Plots I and II cultivated)

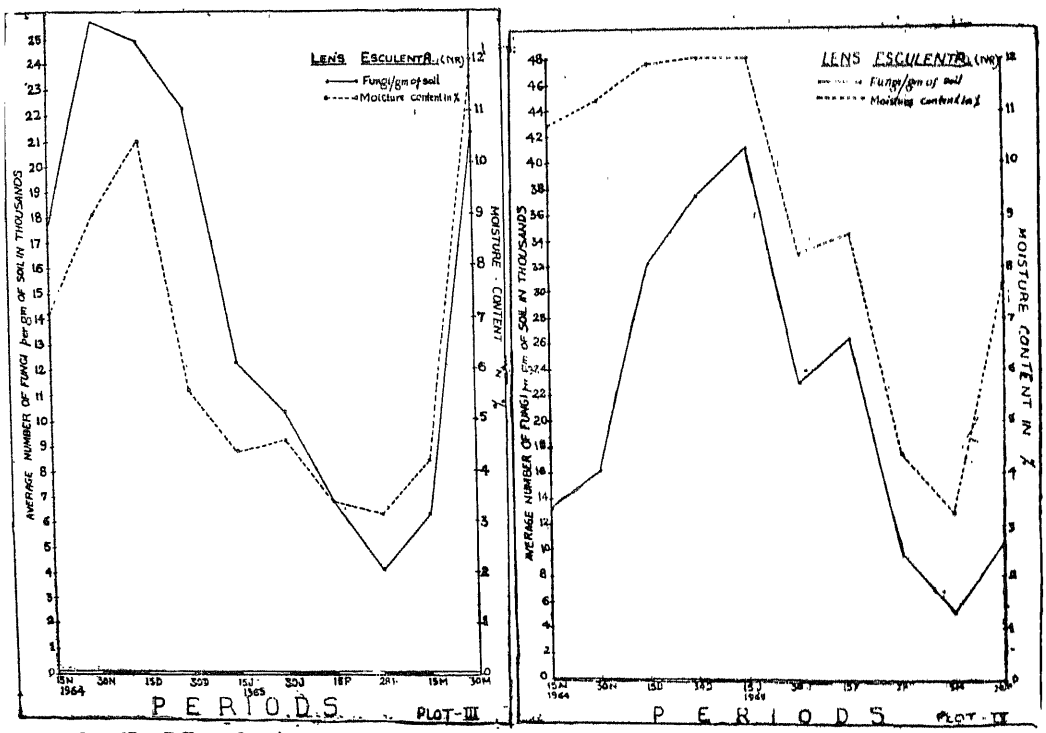
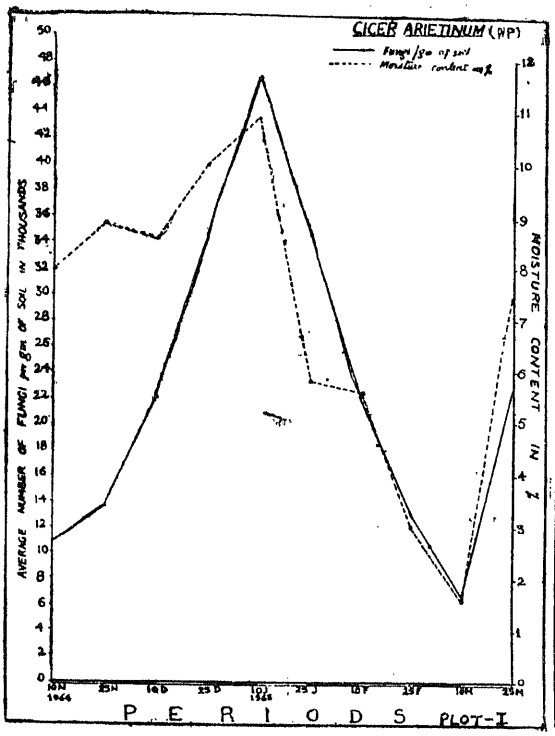


Plate II. Effect of moisture content on the non rhizosphere fungal population of *Lens esculenta* (Plots III and IV experimental)



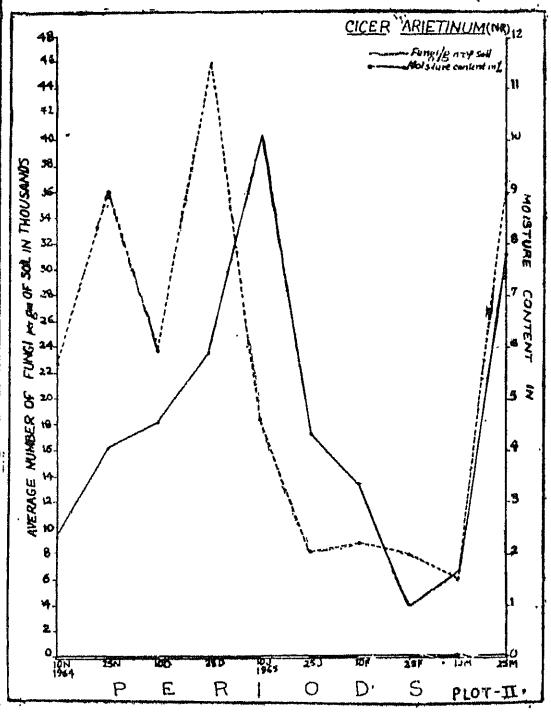
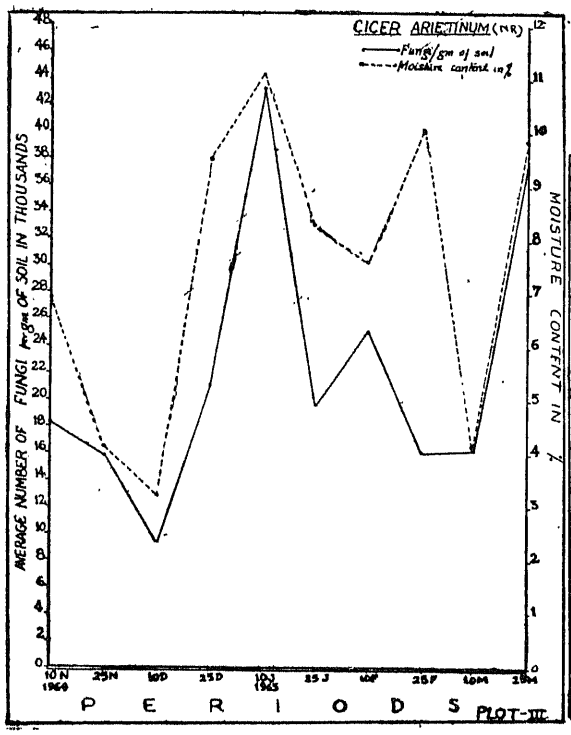


Plate III. Effect of moisutre content on the non-rhizosphere fungal population of *Cicer arietinum* (Plots I and II cultivated)



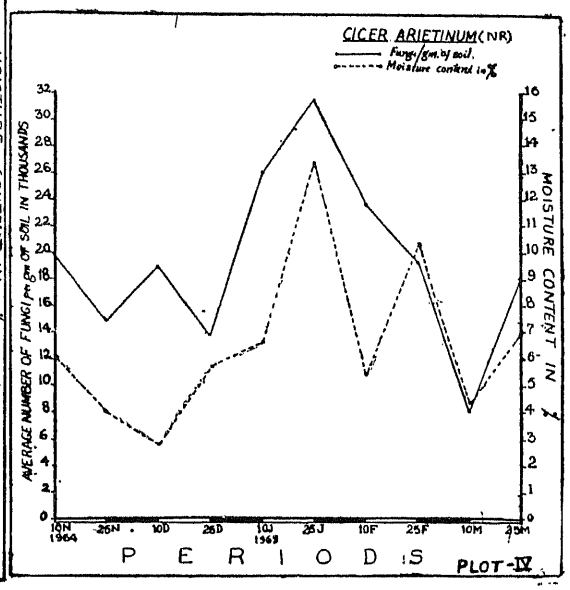


Plate IV. Effect of moisture content on the non-rhizosphere fungal population of *Cicer arietinum* (Plots III and IV experimental)

nigricans and a sterile phycomycetous colony. Except *Thielavia terricola* which sometimes showed high frequency, 5 species, viz. *Chaetomium globosum, C. funicola, Neocosmospora vasinfecta, Aspergillus nidulans* and *Penicillium spicu isporum* had a low frequency. *Trichoderma viride, Aspergillus niger, A. flavus, A. terreus, Penicillium humicola, Paecilomyces fusisporus, Curvularia lunata, Cladosporium herbarum* and *Fusarium nivale* were the dominant forms out of the 42 Deuteromycetes isolated from this plot (Table II).

Fungal population in relation to edaphic factors

As the analysis of the soil samples with regard to W. H. C., pH, organic matter, exchangeable Ca, Mg and phosphate and nitrate at different periods of the growing season does not show any significant differences (Tables III—X), it is presumed that there is no correlation between these factors and the fungal population, although the work of Saksena (1955), Dwivedi (1960) showed direct correlation between W. H. C., carbonate, exchangeable calcium, magnesium, phosphate, nitrate and nitrogen and soil microflora. Ramakrishnan (1953, 1955) also concluded that W. H. C., nitrate, nitrogen and phosphate were directly correlated with the number of fungi, whereas the effect of potassium was not clear. Mishra (1964) observed that W. H. C., organic matter, exchangeable magnesium and phosphate and nitrate affected soil microflora directly, whereas he could not establish any correlation between soil microflora and carbonate and exchangeable calcium.

In the present study, the fungal population showed direct correlation only with the amount of moisture available from soil (Plate I—IV). This work finds support from the work of Waksman (1932, 1952), Dixon (1928), and Jasevoli (1924). Cobb's (1952) work has shown that this correlation may not be closely seen to the same extent in all soils. Under Indian conditions Ghosh and Dutta (1960) and Mishra (1964) observed such a type of direct correlation.

Summary

An investigation into the soil fungal of cultivated fields of *Lens esculenta* and *Cicer arietinum* was undertaken. Fungal population from 4 plots each of the above crop plants was studied. It has been observed that there is not much difference between the fungal flora of the two sets of the plots. However, the dominant fungal species in the *Lens* plots were *Rhizopus nigricans, Mucor luteus, Thielavia terricola, Trichoderma viride, Aspergillus niger, A. flavus, A. terreus, Penicillium humicola, P. funiculosum, Paecilomyces fusisporus, Curvularia lunata, Alternaria humicola, Cladosporium herbarum, Fusarium nivale* and *F. tricinctum*, whereas in *Cicer* plots the dominants were *Mucor hiemalis, Rhizopus nigricans, Choanephora cucurbitarum, Thielavia terricola, Aspergillus nidulans, Aspergillus niger, A. flavus, A. terreus, Penicillium humicola, P. notatum, Paecilomyces fusisporus, Curvularia lunata, Alternaria tenuis, Cladosporium herbarum, Fusarium tricinctum* and *F. nivale*. No clearcut correlation was observed between the water-holding capacity, pH, organic matter, exchangeable Ca, Mg phosphate and nitrate and soil fungal population. Only the moisture content of the soil was directly responsible for fluctuation in the microfungal population. A change in the moisture stutus of the soil has a pronounced effect on the fungal flora.

Acknowledgements

The author wishes to express her gratefulness to Dr. R. Y. Roy, D. I. C. for his valuable guidance ; to Professor R. Misra, F. N. I., for laboratory facilities and to the Council of Scientific and Industrial Research for financial assistance.

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